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ABSTRACT

This course is designed for those requiring an introduction to instruments commonly used in water pollution analyses. Examples are: pH, conductivity, dissolved oxygen meters, spectrophotometers, turbidimeters, carbon analyzer, and gas chromatographs. Students should have a basic knowledge of analytical chemistry. (CO)

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United States
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National Training
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Introduction to Instrumental Analysis of Water Pollutants

Training Manual

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INTRODUCTION TO INSTRUMENTAL ANALYSIS OF WATER POLLUTANTS

This course is designed for those requiring an introduction to instruments commonly used in water pollution analyses. Examples are pH, conductivity, and dissolved oxygen meters, spectrophotometers (infrared, atomic absorption, flame photometry and colorimetric measurements), the turbidimeter, carbon analyzer, and gas chromatographs. Course applicants should have a basic knowledge about analytical chemistry.

At the conclusion of this course the student will be familiar with the various instruments employed to analyze water pollutants in water.

U. S. ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
National Training and Operational Technology Center

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Office of Water Program Operations, U. S. Environmental Protection Agency.

> Due to the limited availability of the manuals it is not appropriate to cite them as technical references in bibliographies or other forms of publication.
>
> References to products and manufacturers are for illustration only; such references do not imply product endorsement by the Office of Water Program Operations, U. S. Environmental Protection Agency.

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations.

This manual will be useful to anyone who has need for information on the subjects covered. However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.

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METHODOLOGY FOR CHEMICAL ANALYSIS OF WATER AND WASTEWATER

I INTRODUCTION

This outline deals with chemical methods which are commonly performed in water quality laboratories. Although a large number of constituents or properties may be of interest to the analyst, many of the methods employed to measure them are based on the same analytical principles. The purpose of this outline is to acquaint you with the principles involved in commonly-used chemical methods to determine water quality.

II PRE-TREATMENTS

For some parameters, a preliminary treatment is required before the analysis begins. These treatments serve various purposes.

A Distillation - To isolate the constituent by heating a portion of the sample mixture to separate the more volatile part(s), and then cooling and condensing the resulting vapor(s) to recover the volatilized portion.

B Extraction - To isolate/concentrate the constituent by shaking a portion of the sample mixture with an immiscible solvent in which the constituent is much more soluble.

C Filtration - To separate undissolved matter from a sample mixture by passing a portion of it through a filter of specified size. Particles that are dissolved in the original mixture are so small that they stay in the sample solution and pass through the filter.

D Digestion - To change constituents to a form amenable to the specified test by heating a portion of the sample mixture with chemicals.

III METERS

For some parameters, meters have been designed to measure that specific constituent or property.

A pH Meters

pH (hydrogen ion concentration) is measured as a difference in potential across a glass membrane which is in contact with the sample and with a reference solution. The sensor apparatus might be combined into one probe or else it is divided into an indicating electrode (for the sample) and a reference electrode (for the reference solution). Before using, the meter must be calibrated with a solution of known pH (a buffer) and then checked for proper operation with a buffer of a different pH value.

B Dissolved Oxygen Meters

Dissolved oxygen meters measure the production of a current which is proportional to the amount of oxygen gas reduced at a cathode in the apparatus. The oxygen gas enters the electrode through a membrane, and an electrolyte solution or gel acts as a transfer and reaction media. Prior to use the meter must be calibrated against a known oxygen gas concentration.

C Conductivity Meters

Specific conductance is measured with a meter containing a Wheatstone bridge which measures the resistance of the sample solution to the transmission of an electric current. The meter and cell are calibrated according to the conductance of a standard solution of potassium chloride at 25°C, measured by a "standard" cell with electrodes one cm square spaced one cm apart. This is why results are called "specific" conductance.

D Turbidimeters

A turbidimeter compares the intensity of light scattered by particles in the sample under defined conditions with the intensity of light scattered by a standard reference suspension.

IV SPECIFIC ION ELECTRODES

Just as the conventional glass electrode for pH develops an electrical potential in response to the activity of hydrogen ion in solution, the specific ion electrode develops an electrical potential in response to the activity of the ion for which the electrode is specific. The potential and activity are related according to the Nernst equation. Simple analytical techniques can be applied to convert activity to an expression of concentration.

These electrodes are used with a pH meter with an expanded mV scale or with a specific ion meter. Two examples are the ammonia and fluoride electrodes.

A Ammonia

The ammonia electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an ammonium chloride internal solution. Ammonia in the sample diffuses through the membrane and alters the pH of the internal solution, which is sensed by a pH electrode. The constant level of chloride in the internal solution is sensed by a chloride selective ion electrode which acts as the reference electrode.

B Fluoride

The fluoride electrode consists of a lanthanum fluoride crystal across which a potential is developed by fluoride ions. The cell may be represented by Ag/Ag Cl, $Cl^-(0.3)$, $F^-(0.001)$ LaF/test solution/SCE/. It is used in conjunction with a standard single junction reference electrode.

V GENERAL ANALYTICAL METHODS

A Volumetric Analysis

Titrations involve using a buret to measure the volume of a standard solution of a substance required to completely react with the constituent of interest in a measured volume of sample. One can then calculate the original concentration of the constituent of interest.

There are various ways to detect the end point when the reaction is complete.

1 Color change indicators

The method may utilize an indicator which changes color when the reaction is complete. For example, in the Chemical Oxygen Demand Test the indicator, ferroin, gives a blue-green color to the mixture until the oxidation-reduction reaction is complete. Then the mixture is reddish-brown.

Several of these color-change titrations make use of the iodometric process whereby the constituent of interest quantitatively releases free iodine. Starch is added to give a blue color until enough reducing agent (sodium thiosulfate or phenylarsine oxide) is added to react with all the iodine. At this end point, the mixture becomes colorless.

2 Electrical property indicators

Another way to detect end points is a change in an electrical property of the solution when the reaction is complete. In the chlorine titration a cell containing potassium chloride will produce a small direct current as long as free chlorine is present. As a reducing agent (phenylarsine oxide) is added to reduce the chlorine, the microammeter which measures the existing direct current registers a lower reading on a scale. By observing the scale, the end point of total reduction of chlorine can be determined because the direct current ceases.

3 Specified end points

For acidity and alkalinity titrations, the end points are specified pH values for the final mixture. The pH values are those existing when common acidity or alkalinity components have been neutralized. Thus acidity is determined by titrating the sample with a standard alkali to pH 8.2 when carbonic acid would be neutralized to $(CO_3)^=$. Alkalinity (except for highly acidic samples) is determined by titrating the sample with a standard acid to pH 4.5 when the carbonate present has been converted to carbonic acid. pH meters are used to detect the specified end points.

B Gravimetric Procedures

Gravimetric methods involve direct weighing of the constituent in a container. An empty container is weighed, the constituent is separated from the sample mixture and isolated in the container, then the container with the constituent is weighed. The difference in the weights of the container before and after containing the constituent represents the weight of the constituent.

The type of container depends on the method used to separate the constituent from the sample mixture. In the solids determinations, the container is an evaporating dish (total or dissolved) or a glass fiber filter disc in a crucible (suspended). For oil and grease, the container is a flask containing a residue after evaporation of a solvent.

C Combustion

Combustion means to add oxygen. In the Total Organic Carbon Analysis, combustion is used within an instrument to convert carbonaceous material to carbon dioxide. An infrared analyzer measures the carbon dioxide.

VI PHOTOMETRIC METHODS

These methods involve the measurement of light that is absorbed or transmitted quantitatively either by the constituent of interest or else by a substance containing the constituent of interest which has resulted from some treatment of the sample. The quantitative aspect of these photometric methods is based on applying the Lambert-Beer Law which established that the amount of light absorbed is quantitatively related to the concentration of the absorbing medium at a given wavelength and a given thickness of the medium through which the light passes.

Each method requires preparing a set of standard solutions containing known amounts of the constituent of interest. Photometric values are obtained for the standards. These are used to draw a calibration (standard) curve by plotting photometric values against the concentrations. Then, by locating the photometric value for the sample on this standard curve, the unknown concentration in the sample can be determined.

A Atomic Absorption

Atomic Absorption (AA) instruments utilize absorption of light of a characteristic wavelength. This form of analysis involves aspirating solutions of metal ions (cations) or molecules containing metals into a flame where they are reduced to individual atoms in a ground electrical state. In this condition, the atoms can absorb radiation of a wavelength characteristic for each element. A lamp containing the element of interest as the cathode is used as a source to emit the characteristic line spectrum for the element to be determined.

The amount of energy absorbed is directly related to the concentration of the element of interest. Thus the Lambert-Beer Law applies. Standards can be prepared and tested and the resulting absorbance values can be used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on this curve to determine the corresponding concentration.

Once the instrument is adjusted to give optimum readings for the element of interest, the testing of each solution can be done in a matter of seconds. Many laboratories wire recorders into their instruments to rapidly transcribe the data, thus conserving time spent on this aspect of the analysis. Atomic absorption techniques are generally used for metals and semi-metals in solution or else solubilized through some form of sample processing. For mercury, the principle is utilized but the absorption of light occurs in a flameless situation with the mercury in the vapor state and contained in a closed glass cell.

B Flame Emission

Flame emission photometry involves measuring the amount of light given off by atoms drawn into a flame. At certain temperatures, the flame raises the electrons in atoms to a higher energy level. When the electrons fall back to a lower energy level, the atoms lose (emit) radiant energy which can be detected and measured.

Again standards must be prepared and tested to prepare a calibration (standard) curve. Then the transmission value of the sample can be located on the curve to determine its concentration.
Many atomic absorption instruments can be used for flame emission photometry. Sodium and potassium are very effectively determined by the emission technique.

However, for many elements, absorption analysis is more sensitive because there are a great number of unexcited atoms in the flame which are available to absorb the radiant energy.

C Colorimetry

Colorimetric analyses involve treating standards which contain known concentrations of the constituent of interest and also the sample with reagents to produce a colored solution. The greater the concentration of the constituent, the more intense will be the resulting color.

The Lambert-Beer Law which relates the absorption of light to the thickness and concentration of the absorbing medium applies. Accordingly, a spectrophotometer is used to measure the amount of light of appropriate wavelength which is absorbed by the same thickness of each solution. The results from the standards are used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on this curve to determine the corresponding concentration.

Many of the metals and several other parameters (phosphorus, ammonia, nitrate, nitrite, etc.) are determined in this manner.

VII GAS-LIQUID CHROMATOGRAPHY

Chromatography techniques involve a separation of the components in a mixture by using a difference in the physical properties of the components. Gas-Liquid Chromatography (GLC) involves separation based on a difference in the properties of volatility and solubility. The method is used to determine algicides, chlorinated organic compounds and pesticides.

The sample is introduced into an injector block which is at a high temperature (e.g. 210°C), causing the liquid sample to volatilize. An inert carrier gas transports the sample components through a liquid held in place as a thin film on an inert solid support material in a column.
Sample components pass through the column at a speed partly governed by the relative solubility of each in the stationary liquid. Thus the least soluble components are the first to reach the detector. The type of detector used depends on the class of compounds involved. All detectors function to sense and measure the quantity of each sample component as it comes off the column. The detector signals a recorder system which registers a response.

As with other instrumental methods, standards with known concentrations of the substance of interest are measured on the instrument. A calibration (standard) curve can be developed and the concentration in a sample can be determined from this graph.

Gas-liquid chromatography methods are very sensitive (nanogram, picogram quantities) so only small amounts of samples are required. On the other hand, this extreme sensitivity often necessitates extensive clean-up of samples prior to GLC analysis.

VIII AUTOMATED METHODS

The increasing number of samples and measurements to be made in water quality laboratories has stimulated efforts to automate these analyses. Using smaller amounts of sample (semi-micro techniques), combining reagents for fewer measurements per analysis, and using automatic dispensers are all means of saving analytical time.
However, the term "automated laboratory procedures" usually means automatic introduction of the sample into the instrument, automatic treatment of the sample to test for a component of interest, automatic recording of data and, increasingly, automatic calculating and print-out of data. Maximum automation systems involve continuous sampling direct from the source (e.g. an in-place probe) with telemetering of results to a central computer.
Automated methods, especially those based on colorimetric methodology, are recognized for several water quality parameters including alkalinity, ammonia, nitrate, nitrite, phosphorus, and hardness.

IX SOURCES OF PROCEDURES

Details of the procedure for an individual measurement can be found in reference books. There are three particularly-recognized books of procedures for water quality measurements.

A Standard Methods(1)

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish "Standard Methods for the Examination of Water and Wastewater." As indicated by the list of publishers, this book contains methods developed for use by those interested in water or wastewater treatment.

B ASTM Standards(2)

The American Society for Testing and Materials publishes an "Annual Book of ASTM Standards" containing specifications and methods for testing materials. The "book" currently consists of 47 parts.

The part applicable to water was formerly Part 23. It is now Part 31, Water.

The methods are chosen by approval of the membership of ASTM and are intended to aid industry, government agencies and the general public. Methods are applicable to industrial waste waters as well as to other types of water samples.

C EPA Methods Manual[3]

The United States Environmental Protection Agency publishes a manual of "Methods for Chemical Analysis of Water and Wastes."

EPA developed this manual to provide methodology for monitoring the quality of our Nation's waters and to determine the impact of waste discharges. The test procedures were carefully selected to meet these needs, using Standard Methods and ASTM as basic references. In many cases, the EPA manual contains completely described procedures because they modified methods from the basic references. Otherwise, the manual cites page numbers in the two references where the analytical procedures can be found.

X ACCURACY AND PRECISION

A Of the Method

One of the criteria for choosing methods to be used for water quality analysis is that the method should measure the desired property or constituent with precision, accuracy, and specificity sufficient to meet data needs. Standard references, then, include a statement of the precision and accuracy for the method which is obtained when (usually) several analysts in different laboratories used the particular method.

B Of the Analyst

Each analyst should check his own precision and accuracy as a test of his skill in performing a test. According to the U. S. EPA Handbook for Analytical Quality Control[4], he can do this in the following manner.

To check precision, the analyst should analyze samples with four different concentrations of the constituent of interest, seven times each. The study should cover at least two hours of normal laboratory operations to allow changes in conditions to affect the results. Then he should calculate the standard deviation of each of the sets of seven results and compare his values for the lowest and highest concentrations tested with the standard deviation value published for that method in the reference book. An individual should have better values than those averaged from the work of several analysts.

To check accuracy, he can use two of the samples used to check precision by adding a known amount (spike) of the particular constituent in quantities to double the lowest concentration used, and to bring an intermediate concentration to approximately 75% of the upper limit of application of the method. He then analyzes each of the spiked samples seven times, then calculates the average of each set of seven results. To calculate accuracy in terms of % recovery, he will also need to calculate the average of the results he got when he analyzed the unspiked samples. Then:

$$\%\ \text{Recovery} = \left[\frac{\text{Avg. of Spiked}}{\begin{matrix}\text{Avg. of}\\ \text{Unspiked}\end{matrix} + \begin{matrix}\text{Amt. of}\\ \text{Spike}\end{matrix}} \right] \times 100$$

Again, the individual's % recovery should be better than the published figure derived from the results of several analysts.

C Of Daily Performance

Even after an analyst has demonstrated his personal skill in performing the analysis, a daily check on precision and accuracy should be done. About one in every ten samples should be a duplicate to check precision and about one in every ten samples should be spiked to check accuracy.

It is also beneficial to participate in interlaboratory quality control programs. The U.S. EPA provides reference samples at no charge to laboratories. These samples serve as independent checks on reagents, instruments or techniques; for training analysts or for comparative analyses within the laboratory. There is no certification or other formal evaluative function resulting from their use.

XI SELECTION OF ANALYTICAL PROCEDURES

Standard sources(1, 2, 3) will, for most parameters, contain more than one analytical procedure. Selection of the procedure to be used in a specific instance involves consideration of the use to be made of the data. In some cases, one must use specified procedures. In others, one may be able to choose among several methods.

A NPDES Permits and State Certifications

A specified analytical procedure must be used when a waste constituent is measured:

1 For an application for a National Pollutant Discharge Elimination System (NPDES) permit under Section 402 of the Federal Water Pollution Control Act (FWPCA), as amended.

2 For reports required to be submitted by dischargers under NPDES.

3 For certifications issued by States pursuant to Section 401 of the FWPCA, as amended.

Analytical procedures to be used in these situations must conform to those specified in Title 40, Chapter 1, Part 136, of the Code of Federal Regulations (CFR). The listings in the CFR usually cite two different procedures for a particular measurement.

The CFR also provides a system of applying to EPA for permission to use methods not cited in the CFR. Approval of alternative methods for nationwide use will be published in the Federal Register.

B Ambient Water Quality Monitoring

For Ambient Water Quality Monitoring, analytical procedures have not been specified by regulations. However, the selection of procedures to be used should receive attention. Use of those listed in the CFR is strongly recommended. If any of the data obtained is going to be used in connection with NPDES permits, or may be used as evidence in a legal proceeding, use of procedures listed in the CFR is again strongly recommended.

C Drinking Water Monitoring

In December, 1975, National Interim Primary Drinking Water Regulations to be effective June 24, 1977 were published in the Federal Register in Title 40, Chapter 1, Subchapter D, Part 141. The publication includes specification of analytical procedures to be used when determining compliance with the maximum contaminant levels of required parameters.

Because of the low concentrations involved in the regulations, there is often just one analytical method cited for each parameter.

Individuals or organizations may apply to EPA for permission to use methods not cited in the above. Approval of alternative methods for nationwide use will be published in the Federal Register.

XII FIELD KITS

Field kits have been devised to perform analyses outside of the laboratory. The kit may contain equipment and reagents for only one test or for a variety of measurements. It may be purchased or put together by an agency to serve its particular needs.

Since such kits are devised for performing tests with minimum time and maximum simplicity, the types of labware and reagents employed usually differ significantly from the equipment and supplies used to perform the same measurement in a laboratory.

A Shortcomings

Field conditions do not accommodate the equipment and services required for pretreatments like distillation and digestion. Nor is it practical to carry and use calibrated glassware like burets and volumetric pipets. Other problems are preparation, transport and storage of high quality reagents, of extra supplies required to test for and remove sample interferences before making the measurement, and of instruments which are very sensitive in detecting particular constituents. One just cannot carry and set up laboratory facilities in the field which are equivalent to stationary analytical facilities.

B Uses

Even though the results of field tests are usually not as accurate and precise as those performed in the laboratory, such tests do have a place in water quality programs.

In situations where only an estimate of the concentrations of various constituents is required, field tests serve well. They are invaluable sources of information for planning a full-scale sampling/testing program when decisions must be made regarding location of sampling sites, schedule of sample collection, dilution of samples required for analysis, and treatment of samples required to remove interferences to analyses.

C NPDES Permits and State Certification

Kit methods are not approved for obtaining data required for NPDES permits or State construction certifications. If one judges that such a method is justifiable for these purposes, he must apply to EPA for permission to use it.

D Drinking Water Monitoring

The DPD test kit for residual chlorine is approved in the December, 1975 Federal Register for monitoring drinking water.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater, 14th Edition. 1976, APHA-AWWA-WPCF, 1015 18th Street, N.W., Washington, D.C. 20036

2 1975 Annual Book of ASTM Standards, Part 31, Water. ASTM, 1916 Race Street Philadelphia, PA 19103.

3 Methods for Chemical Analysis of Water and Wastes. 1974, U. S. EPA, EMSL, Cincinnati, OH 45268.

4 Analytical Quality Control in Water and Wastewater Laboratories. 1972. U. S. EPA, EMSL, Cincinnati, Ohio 45268.

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Descriptors: Analysis, Chemical Analysis, Methodology, Wastewater, Water Analysis

STATISTICS FOR CHEMISTS

I INTRODUCTION

A Statistics may be defined, for our purpose, as a collection of methods which have been developed for handling numerical data pertaining to samples or portions of entire populations.

B The statistical methods with which we will concern ourselves deal with the presentation and analysis of numerical data from samples.

II FREQUENCY

A Definitions

1 Frequency - indicates how many times a particular score occurs in a collection of data

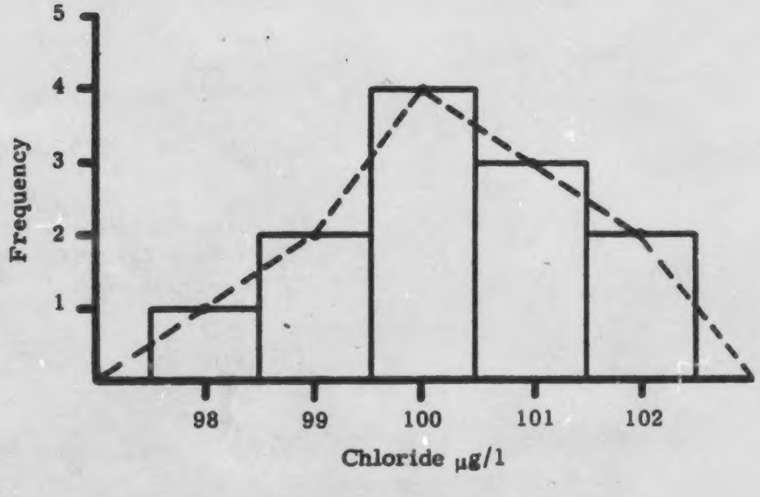
2 Frequency table - a tabular arrangement of data, ranked in ascending or descending order of magnitude, together with the corresponding frequencies

3 Frequency histogram - a set of rectangles having bases on a horizontal axis with centers at the given scores and heights equal to the corresponding frequencies (See Figure 1)

4 Frequency polygon - a line graph of frequencies plotted against scores (can be obtained by connecting midpoints of tops of rectangles in the frequency histogram) (See Figure 1)

Figure 1

Frequency Histogram & Frequency Polygon



B Application

Consider the application of the above definitions to the following set of data, obtained from twelve determinations for chloride in water.

Results (µg/l)

100	101	99
101	100	100
99	102	100
98	101	102

Table 1

Frequency Table

Chloride (µg/l)	Frequency
98	1
99	2
100	4
101	3
102	2

III MEASURES OF CENTRAL TENDENCY

A Definitions

1 Central tendency - the tendency of values to cluster about a particular value in the distribution

2 Mode - that value which occurs most frequently

3 Median - midpoint of an array of scores. If there is an odd number of observations, n, the median is $X_{\frac{n+1}{2}}$ where $X_{\frac{n+1}{2}}$ represents the $\frac{n+1}{2}$ value in the frequency distribution. If there is an even number of observations the median is $\frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2}$, the average of the middle two scores.

4 Mean - arithmetic average of all the values in the sample distribution, denoted by $\bar{X}$. The formula for calculating the sample mean is

$$\bar{X} = \frac{X_1 + X_2 + X_3 \ldots X_n}{n}$$

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

$\bar{X} = \frac{\Sigma X_i}{n}$ where there are n number of values.

B Aids in calculation of the mean

Application of the following two statements can reduce errors and amount of time spent in calculating the mean of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution is equivalent to adding or subtracting the same constant to or from the mean of the distribution. Thus the following formula:

$\bar{X}_c = \bar{X} \pm C$ where the X_i's are the values in the distribution with mean $\bar{X}$, and the $X_i \pm C$'s are the values in the distribution with mean $\bar{X}_c$.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the mean of the distribution by the same constant. Thus the following formulas:

(1) $\bar{X}_c = C\bar{X}$

or

(2) $\bar{X}_c = \frac{\bar{X}}{C}$ where the X_i's are the values in the distribution with mean $\bar{X}$,

and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with mean $\bar{X}_c$

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in Table 1.

1 Mode = 100

2 $$\text{Median} = \frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2} = \frac{X_6 + X_7}{2}$$

$$= \frac{100 + 100}{2} = 100$$

3 $$\text{Mean} = \frac{\Sigma X_i}{n}$$

$$= \frac{98 + 2\,(99) + 4\,(100) + 3\,(101) + 2\,(102)}{12}$$

$$= 100.25$$

4 Aid in Calculation

Consulting Table 1 and observing that the values are in the neighborhood of 100 we might subtract 100 from each score and obtain the following distribution:

Table 2

Frequency Table

Chloride (µg/l)	Frequency
-2	1
-1	2
0	4
1	3
2	2

Denote the mean of the distribution in Table 1 by $\bar{X}_c$. If we add 100 to each score in the distribution in Table 2, we obtain the scores in the distribution in Table 1; likewise if we add 100 to the mean, X, of the distribution in Table 2, we obtain the mean, $\bar{X}_c$, of the distribution in Table 1.

Thus $\bar{X}_c = \bar{X} + 100$

$$\bar{X}_c = \frac{\Sigma X}{n} + 100$$

$$\bar{X}_c = \frac{1(-2) + 2(-1) + 4(0) + 3(1) + 2(2)}{12} + 100$$

$$\bar{X}_c = .25 + 100 = 100.25$$

IV MEASURES OF DISPERSION

A Definitions

1 Dispersion - spread or variability of observations in a distribution

2 Range - the difference between the highest value and the lowest value

$$R = \max - \min$$

3 Average deviation - the sum of the deviations of the values from their mean, without regard to sign, divided by the total number of data values (n)

The formula for calculating the average deviation is:

$$d = \frac{\Sigma\,|X_i - \bar{X}|}{n}$$

4 Average deviation of the mean (D) - the average deviation of individual data items from the mean (d) divided by the square root of the number of data items (n)

The definition of the average deviation of the mean can be expressed by the formula:

$$D = \frac{d}{\sqrt{n}}$$

5 Variance - the sum of the squares of the deviations of the values from their mean divided by the total number of data values (n) minus 1

The definition of the variance can be expressed by the following formula:

$$s^2 = \frac{\Sigma(X_i - \overline{X})^2}{n - 1}$$

6 Standard deviation - the square root of the variance

The definition of the standard deviation can be expressed by the following formula:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n - 1}}$$

However, the formula commonly used because of its adaptability to the hand calculator is the following:

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}}$$ where there are n number of values.

7 Standard deviation of the mean (S) - the standard deviation of individual data items (s) divided by the square root of the number of data items (n)

The definition of the standard deviation of the mean can be expressed by the formula:

$$S = \frac{s}{\sqrt{n}}$$

8 Relative standard deviation - the standard deviation (s) expressed as a fraction of the mean, $\frac{s}{\overline{X}}$

The relative standard deviation is often expressed as a percent. It is then referred to as the coefficient of variation (V):

$$V = \frac{s}{\overline{X}} \times 100 = \%$$

The relative standard deviation is particularly helpful when comparing the precision of a number of determinations on a given substance at different levels of concentration.

B Aids in Calculation

Application of the following statements can reduce errors and amount of time spent in calculating the variance or standard deviation of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution doesn't affect the variance or standard deviation of the distribution.

Thus the following formulas:

(1) $s_c^2 = s^2$

(2) $s_c = s$

where the X_i's are the values in the distribution with variance s^2 and standard deviation s, and the $X_i \pm C$'s are the values in the distribution with variance s_c^2 and standard deviation s_c.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the variance of that distribution by the square of the same constant.

Thus the following formulas:

(1) $s_c^2 = C^2 s^2$

(2) $s_c^2 = \frac{s^2}{C^2}$

where the X_i's are the values in the distribution with variance s^2, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with variance s_c^2.

3 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the standard deviation of that distribution by the same constant.

Thus the following formulas:

(1) $s_c = Cs$

or

(2) $s_c = \frac{s}{C}$

where the X_i's are the values in the distribution with standard deviation s, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with standard deviation s_c.

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in II B, Table 1.

1 Range = 102 - 98 = 4

2 Average deviation - $d = \frac{\Sigma |X_i - \bar{X}|}{n}$

n	X_i	$\lvert X_i - \bar{X} \rvert$	$n\lvert X_i - \bar{X} \rvert$
1	98	2.25	2.25
2	99	1.25	2.50
4	100	.25	1.00
3	101	.75	2.25
2	102	1.75	3.50
	$\bar{X} = 100.25$		11.50

$$d = \frac{\Sigma |X_i - \bar{X}|}{n} = \frac{11.50}{12} = .96$$

3 Average deviation of the mean -

$$D = \frac{d}{\sqrt{n}}$$

Using calculations from number 2,

$$D = \frac{d}{\sqrt{n}} = \frac{0.96}{\sqrt{12}} = \frac{0.96}{3.46} = 0.28$$

4 Variance - $s^2 = \frac{\Sigma (X_i - \bar{X})^2}{n-1}$

n	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	$n(X_i - \bar{X})^2$
1	98	-2.25	5.06	5.06
2	99	-1.25	1.56	3.12
4	100	- .25	.06	.24
3	101	+ .75	.56	1.68
2	102	+1.75	3.06	6.12
				16.22

$$s^2 = \frac{\Sigma (X_i - \bar{X})^2}{n - 1} = \frac{16.22}{11} = 1.47$$

5 Standard deviation - $s = \sqrt{\dfrac{\Sigma X_i^2 - \dfrac{(\Sigma X_i)^2}{n}}{n-1}}$

n	X_i	nX_i	X^2_i	nX^2_i
1	98	98	9604	9604
2	99	198	9801	19602
4	100	400	10000	40000
3	101	303	10201	30603
2	102	204	10404	20808
		1203		120617

$$s = \sqrt{\dfrac{120617 - \dfrac{1203^2}{12}}{11}} = \sqrt{\dfrac{120617 - 120601}{11}}$$

$$s = \sqrt{\dfrac{16}{11}} = 1.21$$

6 Aid in calculation

Recalling that adding or subtracting a constant to each score in the distribution doesn't affect the variance or the standard deviation of the distribution we can simplify the computations by first subtracting 100 from each score in the distribution, thus obtaining the frequency distribution shown in Table 2.

n	X_i-C	n(X_i-C)	(X_i-C)^2	n(X_i-C)^2
1	-2	-2	4	4
2	-1	-2	1	2
4	0	0	0	0
3	1	3	1	3
2	2	4	4	8
		3		17

$$s^2 = \dfrac{\Sigma X_i^2 - \dfrac{(\Sigma X_i)^2}{n}}{n-1}$$

$$s^2 = \dfrac{17 - \dfrac{(3)^2}{12}}{11} = \dfrac{16.25}{11} = 1.48$$

$$s = \sqrt{\dfrac{\Sigma X_i^2 - \dfrac{(\Sigma X_i)^2}{n}}{n-1}} = \sqrt{1.48} = 1.22$$

7 Standard deviation of the mean -

$$S = \dfrac{s}{\sqrt{n}}$$

Using calculations from number 6,

$$S = \dfrac{s}{\sqrt{n}} = \dfrac{1.22}{\sqrt{12}} = \dfrac{1.22}{3.46} = 0.35$$

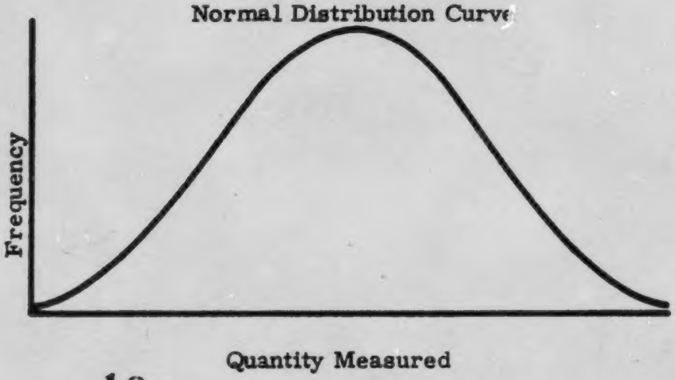
8 Relative standard deviation expressed as a percent (coefficient of variation)

$$V = \dfrac{s}{\overline{X}} \times 100$$

Using calculations from number 6 for $s = 1.22$ and from number 2 for $\overline{X} = 100.25$,

$$V = \dfrac{s}{\overline{X}} = \dfrac{1.22}{100.25} \times 100 = 1.21\%$$

Figure 2
Normal Distribution Curve



V INTRODUCTION TO NORMAL DISTRIBUTION CURVE

A Statistics deals with theoretical curves which are smoother than frequency polygons, obtained from experiments in real life. However, frequency distributions or frequency polygons of experimental data often approximate a mathematical function called the "normal" distribution curve. (See Figure 2)

As shown in Figure 3, the frequency polygon for the 12 determinations for chloride in water is a fairly good approximation of the normal curve. If, however, in the chloride determinations we had obtained 103 instead of 98 and 104 instead of 99 this distribution would not have been a good approximation of the normal curve, as is shown in Figure 4.

Figure 3

Comparison of Normal Curve and Frequency Polygon

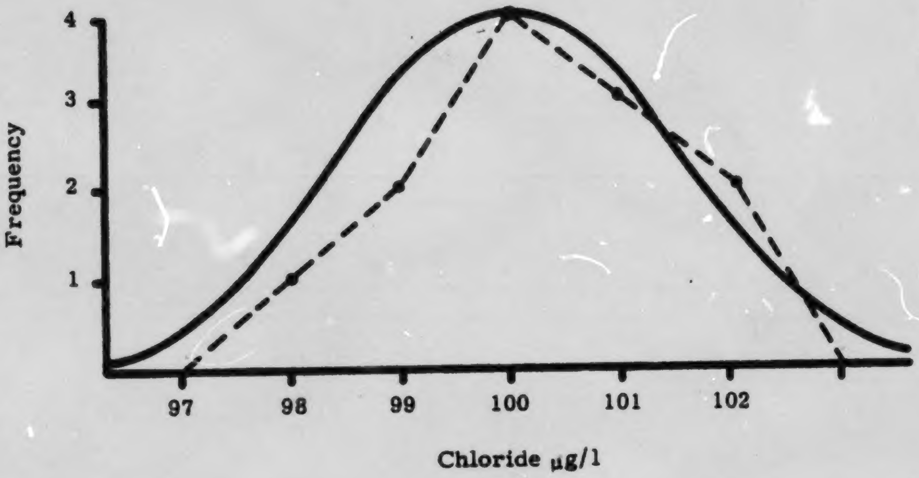
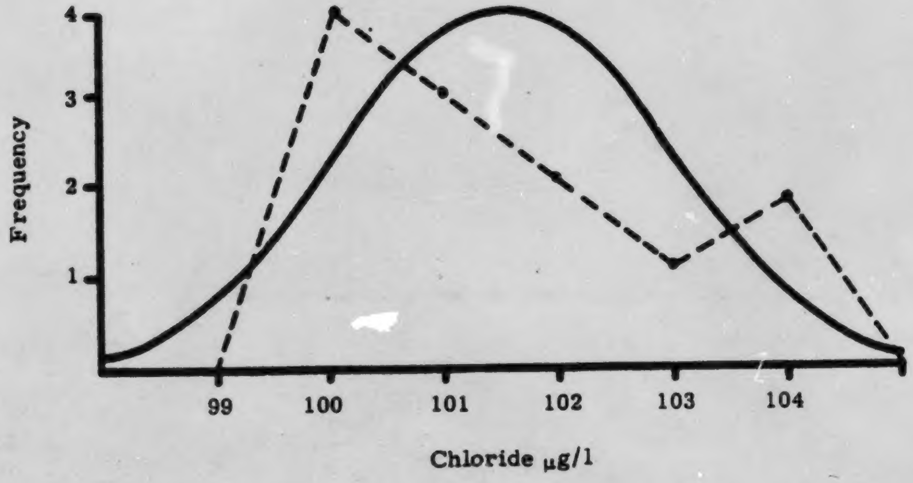


Figure 4

Comparison of Normal Curve and Frequency Polygon



B If a frequency distribution is a good approximation of the normal curve, we can use some facts about the normal curve to give us information about the frequency distribution.

Figure 5 shows the normal distribution in terms of the population mean μ, and the standard deviation of the population σ, and gives the percent of area under the curve between certain points.

Figure 5

Normal Distribution Curve

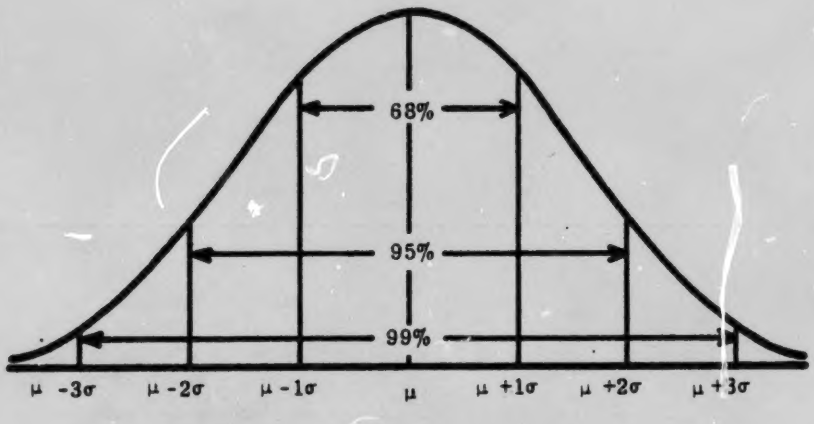
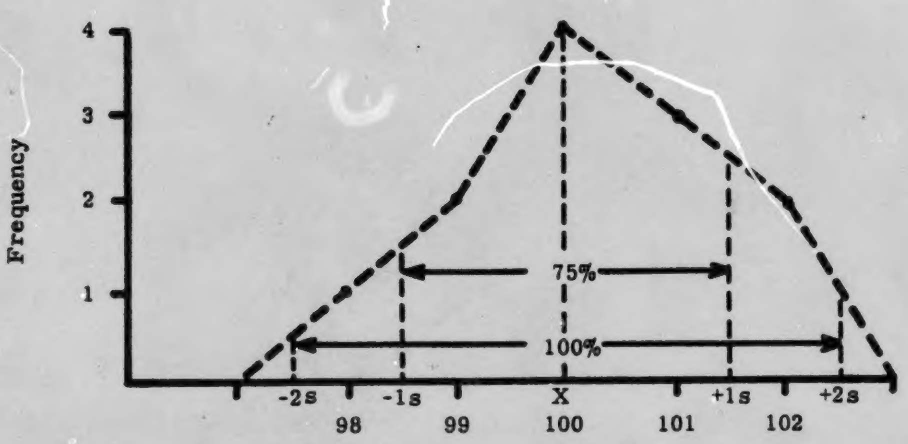


Figure 6

Frequency Distribution Polygon



Chloride μg/l

21

We may check the distribution of sample data to see if it is a "normal" distribution in the following manner. Substitute the value of the sample mean ($\overline{X}$) for the value of the midline and substitute the value of the sample standard deviation (s) for the limits of the value spans where we might expect certain percentages of the data items to occur. Then we can check the number of data items which actually do occur within these value spans.

Figure 6 demonstrates this application using the chloride data values from Table 1. The data values are marked on the horizontal line and the frequency of the occurrence of each value is marked on the vertical. The midline of the distribution is marked at the value of the sample mean ($\overline{X} = 100$, See III C 3). The value of the sample standard deviation ($s = 1.21$, See IV C 5) is used to mark value areas under the curve where different percentages of data values will probably occur. Thus, for the area $\overline{X} \pm 1s$, $\overline{X} - 1s = 98.79$ and $\overline{X} + 1\ s = 101.21$. Therefore, according to the normal distribution curve shown in Figure 5, we might expect about 68% of the data items to have values between 99 and 101. (The values are rounded to whole numbers since the data values are thus recorded).

Consulting Table 1, we find that 75% or 9 of the 12 data items have values in this range. This percentage is shown in Figure 6 by the frequency polygon for the data shown earlier in Figure 3.

Likewise assuming a normal distribution, we would expect 95% of the observations to lie within $\pm 2\sigma$'s from the population mean. In fact, 100% of the observations were within $\pm$ 2 s's from the sample mean.

In both cases the observed percentages are reasonably close to the expected percentages. Other tests exist for determining whether or not a frequency distribution might reasonably be assumed to approximate the normal distribution.

It would be good to become as familiar as possible with the normal distribution since an underlying normal distribution is assumed for many statistical tests of hypothesis.

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This outline was prepared by L. A. Lederer, Statistician, formerly with Analytical Reference Service, Training Program, NCUIH, SEC. Revised by Audrey D.

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Descriptors: Graphic Methods, Quality Control, Statistical Methods, Statistics

INTERLABORATORY QUALITY CONTROL STUDIES

I DEFINITION OF INTERLABORATORY STUDY IN EPA

A It is a between-laboratory evaluation of exact physical, chemical or biological methods which yield a measurement of desired properties.

B It is not an interlaboratory evaluation of materials, reagents, or different test conditions. It is not an initial study of a method, nor a study to develop methods.

II PURPOSES OF INTERLABORATORY STUDY

A Selection of an analytical method.

B Evaluation of an analytical method.

C Evaluation of laboratory and analyst performance.

III PREPARATION FOR INTERLABORATORY STUDY

A Laboratories are in control. Between-replicate deviation will then be very small and uniform in all laboratories.

B Ruggedness testing of a method has been completed by the method developer, or by a single qualified laboratory. Effects of small changes in time, temperature, pH, reagent and sample volume measurements, etc., are known and corrected for.(1)

C All laboratories and analysts are familiar with the method to be tested. Any special equipment or reagents required are known and available., If all analysts are not acquainted with the method, a simple preliminary study is undertaken to accomplish this.(2)

IV INSTRUCTIONS FOR THE STUDY

A Exact method write-ups are provided to each analyst. The technical description of an analytical method is difficult. The language and organization must be complete, yet simple and unambiguous. These characteristics are often conflicting.

Requirements in a method may seem trivial. Yet if these are not recognized and controlled, the entire study can be useless.

B Explicit directions for sample preservation, sample make-up, time limitations, sequence of analyses, etc. , are provided.

C Advance notice of tests is given so that laboratories can integrate the test into their program and realistic deadlines can be established. Once established, deadlines for agreement to participate, for completion of analyses and for reporting are followed closely.

V THE TEST SAMPLES

A The sample is carefully designed to reflect the concentrations found in natural waters and yet be within the best portion of the concentration range for the method.

B Since precision of almost all methods varies with concentration, a comprehensive study includes several levels of concentration.

C Since accuracy of a method is very important, exactly known levels of constituents are added both to distilled and natural waters for testing. Stabilized natural waters are not used as samples.

VI SAMPLE FORM AND CONTAINER

A In the EPA Interlaboratory Program, samples are prepared as concentrates for final dilution at the testing laboratory. Use of concentrates has several advantages. This greatly reduces space requirements and thereby greatly reduces cost of mailing. With use of sealed glass ampules, preservation of concentrates is easily obtained through steam sterilization. Chemical preservatives can be used at fairly high levels in the concentrate and can be removed as interferences by the dilution of the concentrate to final sample volume. Theoretically, a well designed sample properly sealed in glass is stable indefinitely.

B Samples can be prepared as dilute simulated or natural water samples to be analyzed, as is. These samples have an advantage in that they reach the analyst exactly as a routine sample. There is no error in making a dilution and this source of variance in the method is removed.

Some disadvantages of such samples are: the serious logistics problem from storage if a large number of samples is involved; a relatively high cost of mailing; and a limited choice of preservation methods because of the size of containers, the probable use of plastic containers, and only limited testing of water types.

VII INTERLABORATORY STUDY DESIGN

A Sample concentrates are prepared in similar yet different concentrations at each of several levels of analysis.

B True values are calculated from the amount of a constituent added, and are used to measure the accuracy of the study method.

C Multiple constituents are prepared in a sample by related groups of analyses. For example, a mineral and physical sample is analyzed for pH, alkalinity/acidity, specific conductance, total hardness, sulfate, chloride, fluoride, solids, calcium, magnesium, sodium and potassium. A nutrient sample is analyzed for ammonia nitrogen, nitrate nitrogen, Kjeldahl nitrogen, orthophosphate and total phosphorus.

D The analyst dilutes separate aliquots to volume with distilled water and with natural water of his choice.

E Single analyses are made for each parameter. Replicates are of limited value in evaluating a method for routine analyses because of data handling problems and failure to detect significant differences by replication.

F Recoveries are compared for distilled and for natural waters. Recovery from distilled water should indicate method bias. Differences in recoveries from distilled and natural waters should indicate interference by natural water samples.

VIII DATA EVALUATION AND REPORTING

A Rejection of Outliers-Accuracy.

The T-Test is applied to all data using the standard deviation of all data and compared with a T value at the 99% confidence interval. Accuracy is calculated after rejection of this data.

B Precision Measures

A statistical summary is developed for computerized treatment of data as suggested by Larsen(3). Method precision is reported as standard deviation, 95% confidence interval, and coefficient of variation. Method precision is reported for each sample since it varies with concentration.

C **Graphic Display**

Methods are evaluated using two-sample charts described by Youden (4). The values reported by an analyst for a sample pair became the x and y coordinates for a single data point on the chart. Data form ellipses around the true value because of systematic error. Random error is shown as the perpendicular distance from the 45° slope. General scatter indicates poor method precision. Bias is shown by movement of the ellipse away from the true values. Outliers are visible at some distance from the major grouping of data. Examples shown on the following pages are:

Figure 1 -	Outliers,
Figure 2 -	Limited precision and limited accuracy,
Figure 3 -	Negative bias,
Figure 4 -	Positive bias,
Figure 5 -	Systematic error,
Figure 6 -	Good precision and accuracy.

IX INTERLABORATORY STUDY REPORT (5, 6)

A Reports are slanted toward audience.

B All data are coded by laboratory and analyst.

C Glossary of terms is necessary.

D Computer use increases statistical capabilities tremendously. There is a danger of "over-evaluation".

E Increased efficiency in reporting data is achieved by using the computer print-out directly in a report. See Figures 7 and 8.

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This outline was prepared by J. A. Winter, Chief, Method & Performance Evaluation, EMSL, USEPA, Cincinnati, Ohio 45268.

Descriptors: Laboratory Tests, Quality Control, Statistical Methods, Testing Testing Procedures, Error Analysis, Graphic Methods

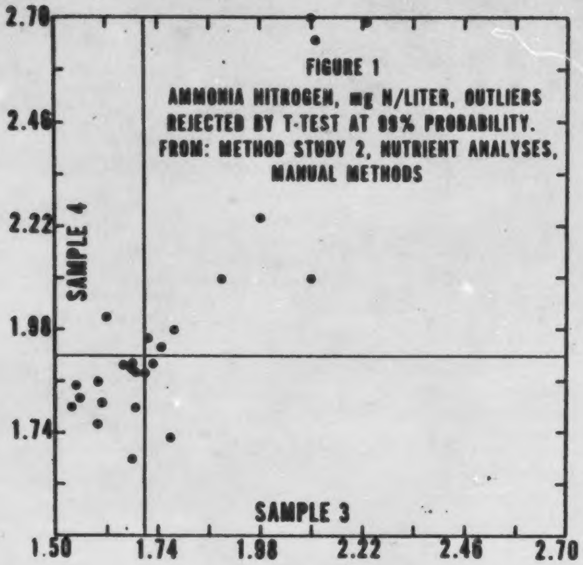


FIGURE 1
AMMONIA NITROGEN, mg N/LITER, OUTLIERS REJECTED BY T-TEST AT 99% PROBABILITY. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

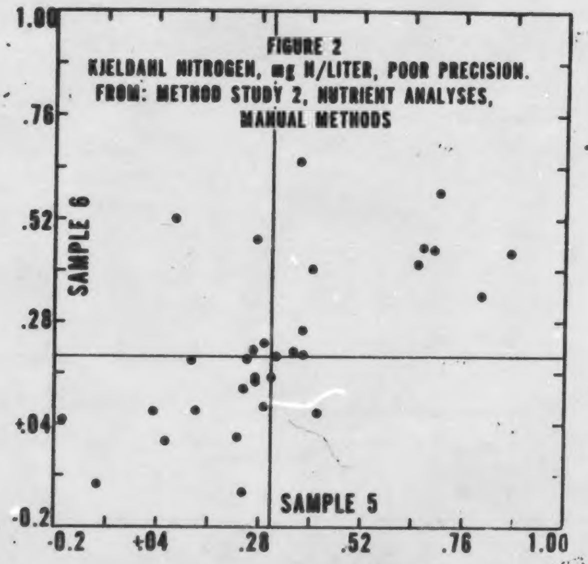


FIGURE 2
KJELDAHL NITROGEN, mg N/LITER, POOR PRECISION. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

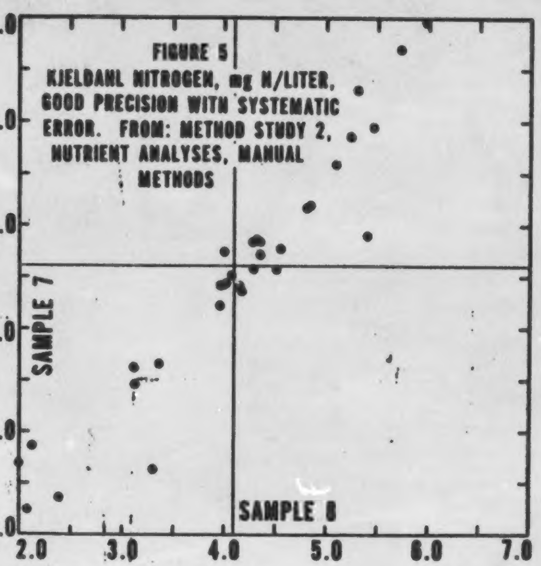


FIGURE 5
KJELDAHL NITROGEN, mg N/LITER, GOOD PRECISION WITH SYSTEMATIC ERROR. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

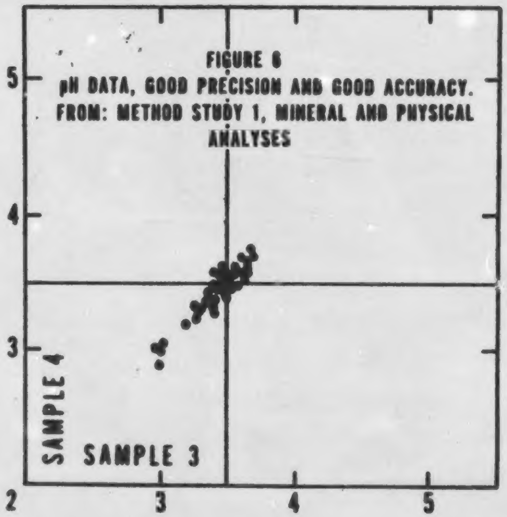


FIGURE 6
pH DATA, GOOD PRECISION AND GOOD ACCURACY. FROM: METHOD STUDY 1, MINERAL AND PHYSICAL ANALYSES

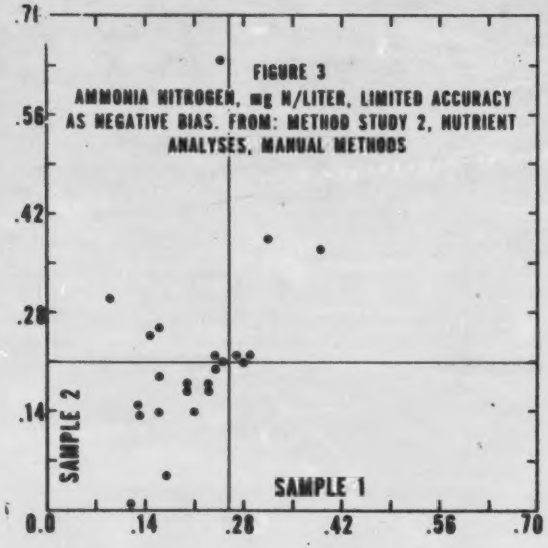


FIGURE 3
AMMONIA NITROGEN, mg N/LITER, LIMITED ACCURACY AS NEGATIVE BIAS. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

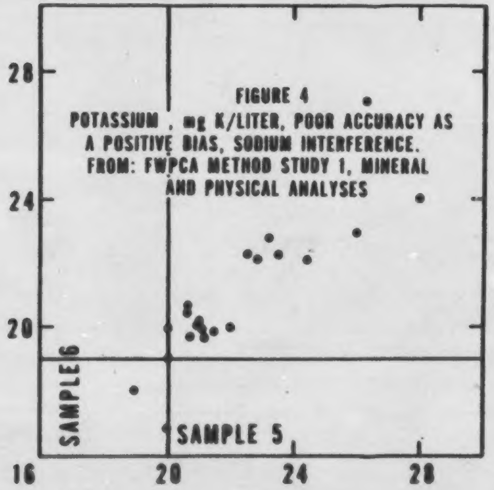
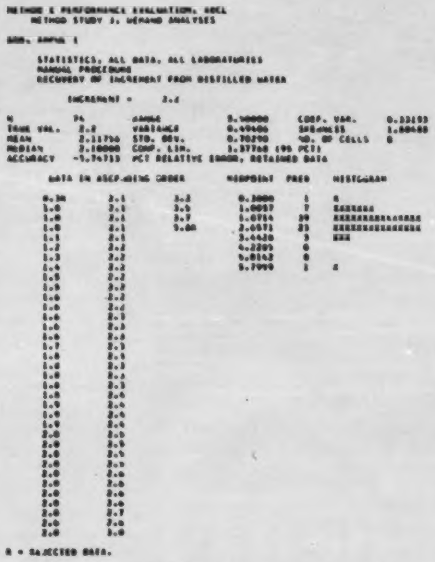


FIGURE 4
POTASSIUM , mg K/LITER, POOR ACCURACY AS A POSITIVE BIAS, SODIUM INTERFERENCE. FROM: FWPCA METHOD STUDY 1, MINERAL AND PHYSICAL ANALYSES



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METHOD & PERFORMANCE EVALUATION, AQCL
     METHOD STUDY 3, DEMAND ANALYSES

BOD, SAMPLE 1

     STATISTICS, ALL DATA, ALL LABORATORIES
     MANUAL PROCEDURE
     RECOVERY OF INCREMENT FROM DISTILLED WATER
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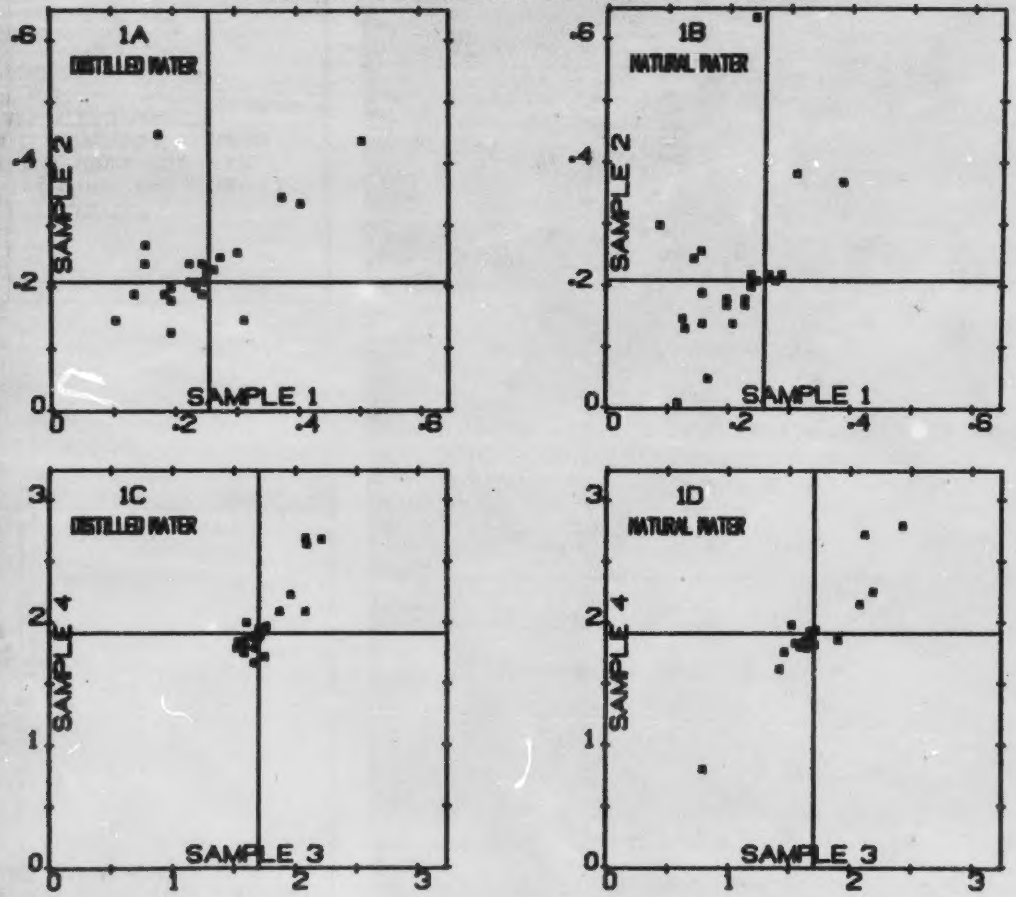
FIGURE 7
BOD DATA, STATISTICAL SUMMARY. PREPARED BY COMPUTER FOR DIRECT USE IN REPORT.
FROM: METHOD RESEARCH STUDY 3, DEMAND ANALYSES

FIGURE 8
AMMONIA NITROGEN DATA, YOUDEN'S TWO-SAMPLE CHARTS.
PREPARED FOR REPORT BY COMPUTER PLOTTER



SPECIFIC CONDUCTANCE

I INTRODUCTION

An electrical conductivity measurement of a solution determines the ability of the solution to conduct an electrical current. Very concentrated solutions have a large population of ions and transmit current easily or with small resistance. Since resistivity is inversely related to conductivity $K = \frac{1}{R}$, a very concentrated solution has a very high electrical conductivity.

Electrical conductivity is determined by transmitting an electrical current through a given solution, using two electrodes. The resistance measured is dependent principally upon the ionic concentration, ionic charge, and temperature of the solution although electrode characteristics (surface area and spacing of electrodes) is also critical. Early experiments in standardizing the measurement led to construction of a "standard cell" in which the electrodes were spaced exactly 1 cm and each had a surface area of 1 cm^2. Using this cell, electrical conductivity is expressed as "Specific Conductance". Modern specific conductance cells do not have the same electrode dimensions as the early standard cell but have a characteristic electrode spacing/area ratio known as the "cell constant".

$$K_{sp} = \frac{1}{R} \times \frac{\text{distance (cm)}}{\text{area (cm}^2)}, \quad K_{sp} = \frac{1}{R} \times k$$

k = cell constant

Specific conductance units are Mhos/cm or reciprocal ohms/cm. Most natural, fresh waters in the United States have specific conductances ranging from 10 to 1,000 micromhos/cm. (1 micromho = 10^{-6} mho).

II CONDUCTIVITY INSTRUMENTS

Nearly all of the commercial specific conductance instruments are of a bridge circuit design, similar to a Wheatstone Bridge. Null or balance is detected either by meter movement, electron "ray eye" tubes, or headphones. Since resistance is directly related to temperature, some instruments have automatic temperature compensators, although inexpensive models generally have manual temperature compensation.

Conductivity instruments offer direct specific conductance readout when used with a cell "matched" to that particular instrument.

Electrodes within the cell may become damaged or dirty and accuracy may be affected; therefore, it is advisable to frequently check the instrument readings with a standard KCl solution having a known specific conductance.

III CONDUCTIVITY CELLS

Several types of conductivity cells are available, each having general applications. Dip cells are generally used for field measurement, flow cells for measurement within a closed system, and pipet cells for laboratory use. Many modifications of the above types are available for specialized laboratory applications; the Jones cells and inductive capacitance cells are perhaps the most common.

Examples of various cell ranges for the RB3 - Industrial Instruments model (0-50 micromhos/cm scale range) are in Table 1.

Cell Number	Relative Conductivity Value	Maximum range micromhos/cm	Most accurate range micromhos/cm
Cel VSO2	1	0 - 50	2 - 30
Cel VS2	10	0 - 500	20 - 300
Cel VS2O	100	0 - 5000	200 - 3000

Table 1

IV Computation of Calibration Constant

A calibration constant is a factor to which scale readings must be multiplied by compute specific conductance.

$K_{sp} = cM$

where K_{sp} = actual specific conductance

c = calibration constant

M = meter reading

For example, a 0.001 N KCl solution (147 micromhos/cm standard) may show a scale reading of 147.

$$147 = c\ 147,\ c = \frac{147}{147} = 1.00$$

In this case the cell is perfectly "matched" to the instrument, the calibration constant is 1.00, and the scale reading represents actual specific conductance. A variety of cells, each covering a specific range, may be used with any one instrument. However, a calibration constant for each cell must be computed before solutions of unknown specific conductance can be determined.

V RELATIONSHIP OF SPECIFIC CONDUCTANCE TO IONIC CONCENTRATION

Natural water consists of many chemical constituents, each of which may differ widely in ionic size, mobility, and solubility. Also, total constituent concentration and proportions of certain ions in various natural waters range considerably. However, it is surprising that for most natural waters having less than 2,000 mg/l. dissolved solids, dissolved solids values are closely related to specific conductance values, ranging in a ratio of .62 to .70. Of course this does not hold true for certain waters having considerable amounts of nonionized soluble materials, such as organic compounds and nonionized, colloidal inorganics.

Properties of some inorganic ions in regard to electrical conductivity are shown below:

Ion	Micromhos/cm per meq/l conc.
Calcium	52.0
Magnesium	46.6
Sodium	48.9
Potassium	72.0
Bicarbonate	43.6
Carbonate	84.6
Chloride	75.9

VI ESTIMATION OF CONSTITUENT CONCENTRATIONS

Generally speaking, for waters having a dissolved solids concentration of less than 1,000 mg/l, calcium and magnesium (total hardness), sodium, bicarbonate and carbonate (total alkalinity), and sulfate are the principal or most abundant ions, representing perhaps 90-99% of the total ionic concentration of the water. Specific conductance, total hardness and total alkalinity are all simple and expedient measurements which can be performed in the field. Therefore, the remaining principal ions are sodium and sulfate, and concentrations of these can be estimated by empirical methods. For example, we find that a certain water has:

K_{sp} = 500 micromhos/cm
Total Hardness = 160 mg/l or 3.20 meq/l
Total Alkalinity = 200 mg/l or 3.28 meq/l, as bicarbonate.

Next we multiply the specific conductance by *0.011 (500 × 0.011 = 5.50) to estimate the total ionic concentration in meq/l.

Cations (meq/l)		Anions (meq/l)	
Calcium	3.20	Carbonate	0.00
Magnesium		Bicarbonate	3.28
Sodium	5.50-3.20 = 2.30	Sulfate	5.50-3.28 = 2.22
Total Cations	5.50	Total Anions	5.50

---

* This factor may vary slightly for different waters

Realizing that several variables are involved in empirical analysis, application rests entirely upon testing the formula with previous complete laboratory analyses for that particular water. If correlation is within acceptable limits, analytical costs may be substantially reduced. Empirical analysis can also be used in determination of proper aliquots (dilution factor) necessary for laboratory analysis.

Records of laboratory chemical analysis may indicate that a particular stream or lake shows a characteristic response to various streamflow rates or lake water levels. If the water's environment has not been altered and water composition responds solely to natural causes, a specific conductivity measurement may be occasionally used in substitution for laboratory analyses to determine water quality. Concentration of individual constituents can thus be estimated from a specific conductance value.

VII APPLICATIONS FOR SPECIFIC CONDUCTANCE MEASUREMENTS

A Laboratory Operations(2)

1 Checking purity of distilled and deionized water

2 Estimation of dilution factors for samples

3 Quality control check on analytical accuracy

4 An electrical indicator

B Agriculture

1 Evaluating salinity

2 Estimating Sodium Adsorption Ratio

C Industry(3)

1 Estimating corrosiveness of water in steam boilers

2 Efficiency check of boiler operation

D Geology

1 Stratigraphic identification and characterization

- a geological mapping
- b oil explorations

E Oceanography

1 Mapping ocean currents

2 Estuary studies

F Hydrology

1 Locating new water supplies

- a buried stream channels (See Fig. 1)
- b springs in lakes and streams (See Fig. 2)

2 Detection and regulation of sea water encroachment on shore wells

G Water Quality Studies

1 Estimation of dissolved solids(2) (See Section V, also Fig. 3)

2 Empirical analysis of constituent concentrations (See Section VI, also reference 2)

3 Quality control check for salt water conversion studies

4 Determination of mixing efficiency of streams (See Fig. 4)

5 Determination of flow pattern of polluted currents (See Fig. 4)

6 Identification of significant fluctuations in industrial wastewater effluents.

7 Signal of significant changes in the composition of influents to waste treatment plants

FIGURE 1

DETECTION OF BURIED STREAM CHANNELS

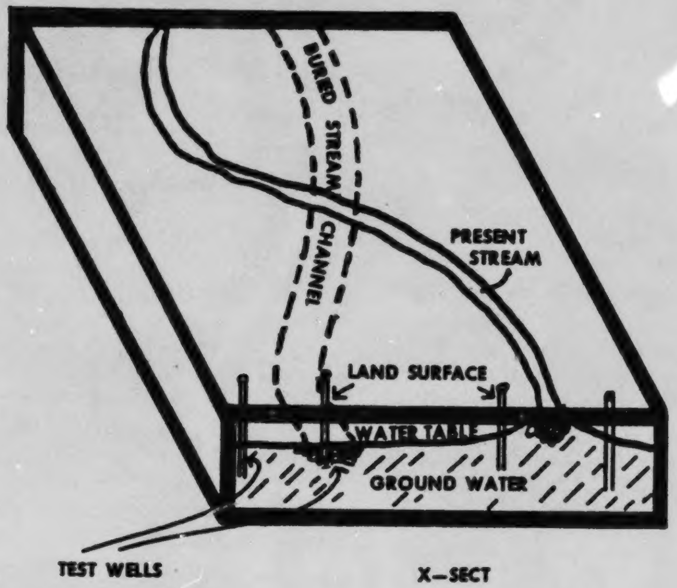
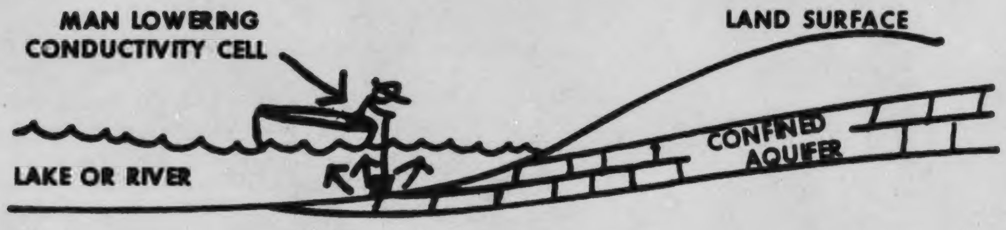

BURIED STREAM CHANNEL
PRESENT STREAM
LAND SURFACE
WATER TABLE
GROUND WATER
TEST WELLS
X—SECT

FIGURE 2

DETECTION OF SPRINGS IN LAKES AND STREAMS


MAN LOWERING CONDUCTIVITY CELL
LAND SURFACE
LAKE OR RIVER
CONFINED AQUIFER

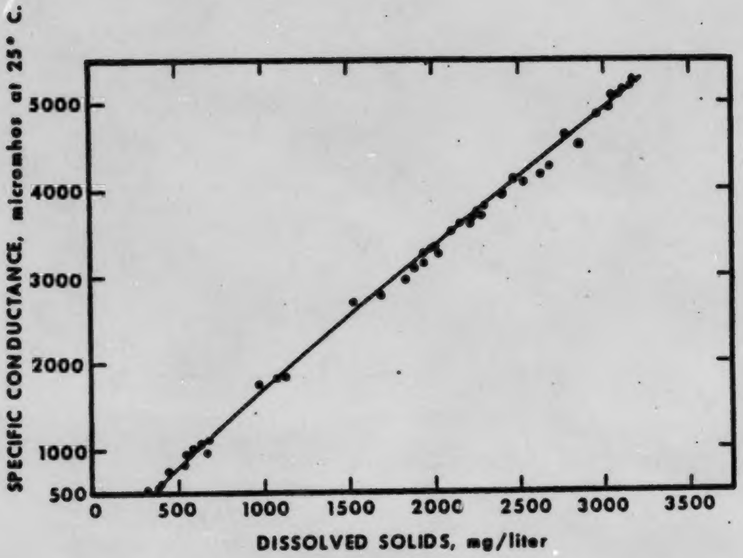


Figure 3. SPECIFIC CONDUCTANCE AND DISSOLVED SOLIDS IN COMPOSITES OF DAILY SAMPLES. , GILA RIVER AT BYLAS, ARIZONA, OCTOBER 1, 1944 TO SEPTEMBER 30, 1944.

Geological Survey Water-Supply Paper 1473.

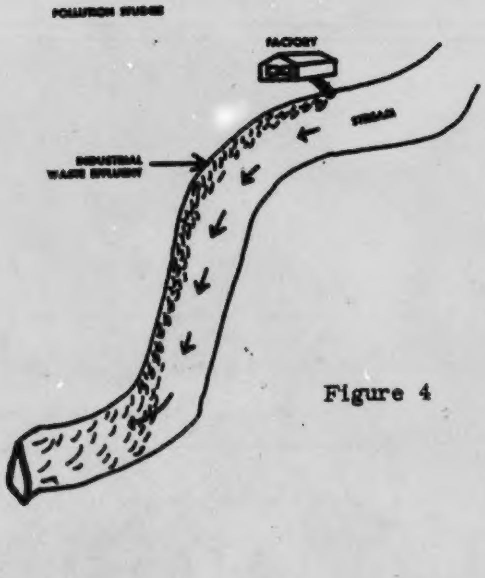

POLLUTION STUDIES
FACTORY
STREAM
INDUSTRIAL
WASTE EFFLUENT

Figure 4

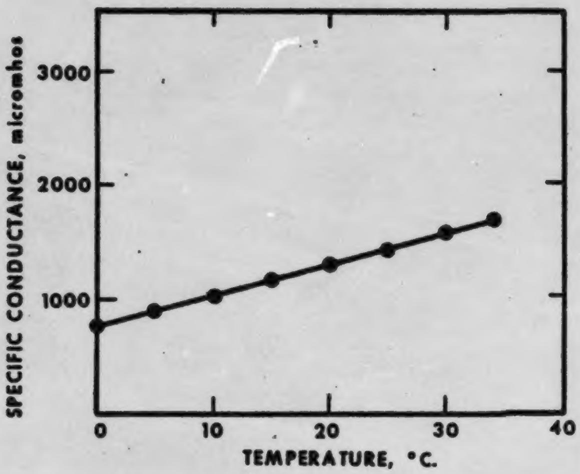


Figure 5. SPECIFIC CONDUCTANCE OF A 0.01 NORMAL SOLUTION OF POTASSIUM CHLORIDE AT VARIOUS TEMPERATURES.

Geological Survey Water-Supply Paper 1473.

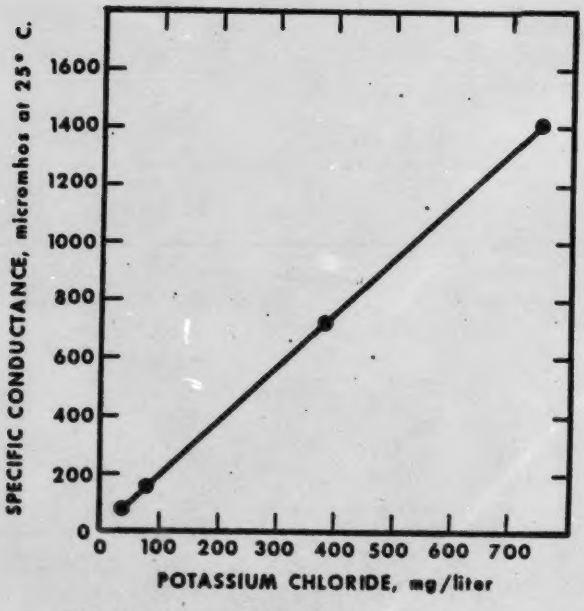


Figure 6. SPECIFIC CONDUCTANCE OF POTASSIUM CHLORIDE SOLUTIONS.

Geological Survey Water-Supply Paper 1473

VIII NPDES METHODOLOGY

A The Federal Register "List of Approved Test Procedures" for NPDES requirements specifies that specific conductance be measured with a self-contained conductivity meter, Wheatstone bridge type.(1) (2) (3)

Temperature directly affects specific conductance values (see Fig. 5). For this reason, samples should preferably be analyzed at 25°C. If not, temperature corrections should be made and results reported as µmhos/cm at 25°C.

1 The instrument should be standardized using KCl solutions. (See Fig. 6)

2 It is essential to keep the conductivity cell clean.

B The EPA manual specifies using the procedure as described in Standard Methods(2) or in ASTM Standards(3). These are approved in 40 CFR136 for NPDES Report purposes.

C Precision and Accuracy(1)

Forty-one analysts in 17 laboratories analyzed 6 synthetic water samples containing the following K_{sp} increments of inorganic salts: 100, 106. 808, 848, 1640 and 1710 micromhos/cm.

The standard deviation of the reported values was 7.55, 8.14, 66.1, 79.6, 106 and 119 µmhos/cm respectively.

The accuracy of the reported values was -2.0, -0.8, -29.3, -38.5, -87.9 and -86.9 µmhos/cm bias respectively.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, Ohio 45268, 1974.

2 Standard Methods for the Examination of Water and Wastewater, APHA-AWWA-WPCF, 14th Edition, 1976.

3 ASTM Annual Book of Standards, Part 31, 1975.

This outline was prepared by John R. Tilstra, Chemist, National Eutrophication Research Program, Corvallis, Oregon with additions by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Concentration, Conductivity, Dissolved Solids, Electrical Conductance, Ions, Physical Properties, Salinity, Sodium, Specific Conductivity, Sulfates, Water Analysis, Water Supplies.

CALIBRATION AND USE OF A CONDUCTIVITY METER

I EQUIPMENT AND REAGENTS

A Equipment

1 Solu Bridge conductivity meters

2 Probes

   a Cell VSO2

   b Cell VS2

   c Cell VS2O

3 Thermometers

4 400 ml beakers

B Reagents

1 Standard KC1 solutions

Normality of KC1 Solution	Specific Conductance micromhos/cm.
0.0001	14.9
0.001	147.0
0.01	1413.0
0.1	12900.0

2 Distilled water

II CHECKING THE INSTRUMENT

A The measurement of specific conductivity as presented in sections II and III is written for one type of conductivity meter and probe.

B A battery check is made by depressing the Battery Check switch, and at the same time pressing the on-off button. The meter needle should deflect to the right (positive) and come to rest in the green zone.

C Place a 10,000 ohm resistor in the holes of the electrical contacts on the meter. Turn the temperature knob to read 25°C. Depress the on-off button and bring the meter needle to a reading of 0 by turning the specific conductance switch. The specific conductance reading should be approximately 200 micromhos/cm.

III DETERMINATION OF THE CALIBRATION CONSTANT

A Determine the temperature of the standard KC1 solutions and move the temperature knob to that value.

B Connect probe Cell VS02 to the conductivity meter.

C Rinse the probe in the beaker of distilled water, wipe the excess water with a kimwipe and place probe in the first beaker of KC1 solution (0.0001 N).

D Make certain the cell is submerged to a point at least 1/2 inch above the air hole and that no entrapped air remains. The cell should also be at least 1/2 inch from the inside walls of the flask.

E Press and hold down the ON-OFF button, simultaneously rotating the main scale knob until the meter reads zero. Release the button. (If the meter needle remains off scale or cannot be nulled, discontinue testing in that solution.)

F Record the scale reading in Table 1 and proceed to KC1 solutions 0.001N, 0.01N, 0.1 N using Steps C, D and E.

G Repeat steps C through F using the VS2, then the VS20 probe.

H Compute the cell calibration constant-a factor by which scale readings must be multiplied to compute specific conductance:

$K_{sp} = cM$

where K_{sp} = actual specific conductance,

c = calibration constant

M = meter reading

(continued next page)

TABLE 1 DATA FOR CALIBRATION CONSTANTS

Probe	Cell VSO2				Cell VS2				Cell VS20			
KCl Solutions	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N	0.0001N	0.001N	0.01N	0.1N
Test # 1												
Cell Constant												

For each cell, calculate the cell constant by using the meter reading closest to the 400 - 600 range. The known specific conductance for the corresponding KCl solution can be found in IB Reagents. Record the cell constants on Table I.

IV DETERMINATION OF K_{sp} FOR SAMPLES

Obtain meter readings, M, for samples A, B and C using Section III C, D and E. Record M in Table 2. See Table I for the appropriate cell constant, c, to calculate K_{sp} for each sample where $K_{sp} = cM$. Record results in Table 2.

V EPA METHODOLOGY

The current EPA Manual(1) specifies using the procedures found in References 2 and 3. These procedures have been adapted for this laboratory session and all are approved in 40CFR136 for NPDES report purposes.

ACKNOWLEDGMENT

This outline contains certain portions of previous outlines by Messrs. J. W. Mandia, and J. R. Tilstra.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, USEPA, AQCL, Cincinnati, OH 45268, 1974.

2 Standard Methods for the Examination of Water and Wastewater, 14th Edition. 1976.

3 Book of ASTM Standards, Part 31, 1975.

This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational Technology Center, and revised by Audrey D. Kroner, also with National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analytical Techniques, Conductivity, Electrical Conductance, Specific Conductivity, Water Analysis.

TABLE 2. SPECIFIC CONDUCTIVITY TESTS

Sample	A			B			C		
Probe	Cell VSO2	Cell VS2	Cell VS2O	Cell VSO2	Cell VS2	Cell VS2O	Cell VSO2	Cell VS2	Cell VS2O
1									
Cell Constant									
Sp. Cond. μmhos/cm									

DISSOLVED OXYGEN

Factors Affecting DO Concentration in Water

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference between an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G Minimum allowable DO concentrations are specified in all Water Quality standards.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

1) Area of the air-water interface.

2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

The first depends upon the surface area of the air bubbles in the water or water drops in the air; the second depends upon the mixing energy in the liquid. If diffusors are placed in a line along the wall, dead spots may develop in the core. Different diffusor placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

b Other variables in oxygen transfer include:

3) Oxygen deficit in the liquid.

4) Oxygen content of the gas phase.

5) Time.

If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about 95%. For example: An oxygen-depleted sample often will pick up significant DO during DO testing; changes are unlikely with a sample containing equilibrium amounts of DO.

c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

e One liter of air at room temperature contains about 230 mg of oxygen. A 5 gal carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30° C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/1 under one atmospheric pressure of air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20° C).

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

   a Photosynthesis occurs only under the influence of adequate light intensity.

   b Respiration of alga is continuous.

   c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

a Resulting benthic deposits continue to respire as bed loads.

b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer to the bed load vicinity.

c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia.

REFERENCE

1 Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio, 45268, 1974.

This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, and revised by Charles R. Feldmann, Chemist, National Training & Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Aeration, Aerobic Conditions, Air-Water Interfaces, Anaerobic Conditions, Benthos, Biological Oxygen Demand, Dissolved Oxygen, Water Pollution, Water Quality

DISSOLVED OXYGEN
Determination by the Winkler Iodometric Titration - Azide Modification

This method is applicable for use with most wastewaters and streams that contain nitrate nitrogen and not more than 1 mg/1 of ferrous iron. Other reducing or oxidizing materials should be absent. If 1 ml of fluoride solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg/1 ferric iron.

The azide modification is not applicable under the following conditions: (a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; (b) samples high in suspended solids; (c) samples containing organic substances which are readily oxidized in a highly alkaline solution, or which are oxidized by free iodine in an acid solution; (d) untreated domestic sewage; (e) biological flocs; and (f) where sample color interferes with endpoint detection. In instances where the azide modification is not applicable, the DO probe should be used.

A Reactions

1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.

$$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4 \qquad (a)$$

$$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2 \qquad (b)$$

$$MnO(OH)_2 + 2\ H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O \qquad (c)$$

$$Mn(SO_4)_2 + 2\ KI \rightarrow MnSO_4 + K_2SO_4 + I_2 \qquad (d)$$

$$I_2 + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaI \qquad (e)$$

2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

a $O_2 \rightarrow MnO(OH)_2 \rightarrow Mn(SO_4)_2 \rightarrow I_2 \rightarrow$ Thiosulfate (thio) or phenylarsine oxide (PAO) titration.

b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.

3 The first conversion, $O_2 \rightarrow MnO(OH)_2$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.

a The manganous salt can react with oxygen only in a highly alkaline media.

b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).

c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross excess of reagents is used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6 times, allowing the floc to settle until there is clear liquid above the floc, repeating the inversion, & allowing the floc to settle about two-thirds of the way down in the bottle. The reaction is rapid; contact is the principal problem in the two phase system.

d If the alkaline floc is white, no oxygen is present.

4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.

5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.

a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

b Phenylarsine oxide solutions are more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

6 For practical purposes the DO determination scheme involves the following operations.

a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

b To the filled bottle:

1) Add $MnSO_4$ reagent (2 ml)

2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle until there is clear liquid above the floc, repeat the inversion, & allow the floc to settle about two-thirds of the way down in the bottle. Highly saline & other test waters may settle very slowly. In this case, allow some reasonable time (e.g. 2 min.) for completion of the reaction.

c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix until the precipitate dissolves.

d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 normal thiosulfate. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality; e. g.,

1) For a 200 ml sample, use 0.025 N Thio

2) For a 100 ml sample, use 0.0125 N Thio

7 The addition of the first two DO reagents, ($MnSO_4$ and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate $MnO(OH)_2$. Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300} \times 100$ or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.

8 Reagent preparation and procedural details can be found in reference 1.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as:

A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by:

1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.

2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.

3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.

4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl or H_2O_2 may raise the DO titration while H_2S, & SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or $SO_3^{=}$ may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from KI while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

1 These reactions may be represented as follows:

$$\begin{array}{ll} \underset{\uparrow}{2HNO_2} + 2\ HI \rightarrow I_2 + 4H_2O + \underset{\downarrow}{N_2O_2} & (a) \\ 2HNO_2 \leftarrow + 1/2O_2 + H_2O + N_2O_2 & (b) \end{array}$$

These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

2 Sodium azide (NaN_3) reacts with nitrite under acid conditions to form a combination of $N_2 + N_2O$ which effectively blocks the cyclic reaction by converting the HNO_2 to noninterfering compounds of nitrogen.

3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of NO_2 N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe^{+++} may confuse endpoint detection if present in significant quantities.

F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.

G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

REFERENCE

1 Methods for Chemical Analysis of Water & Wastes. U. S. Environmental Protection Agency, Environment Monitoring & Support Laboratory, Cincinnati, Ohio 45268, 1974.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia. Review and comments by C. R. Hirth and R. L. Booth are greatly appreciated.

This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, and revised by C. R. Feldmann, Chemist, National Training & Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Dissolved Oxygen, Oxygen, Water Analysis

DISSOLVED OXYGEN
DETERMINATION BY ELECTRONIC MEASUREMENT

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^-$ The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^o - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$2H_2 + O_2 \rightleftarrows 2H_2O$

$2H_2 - 4e \rightleftarrows 4H^{+1}$ hydrogen oxidized

$O_2 + 4e \rightleftarrows 2O^{-2}$ oxygen reduced

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I. C. 3, the reduction of oxygen occurs at the cathode.

8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

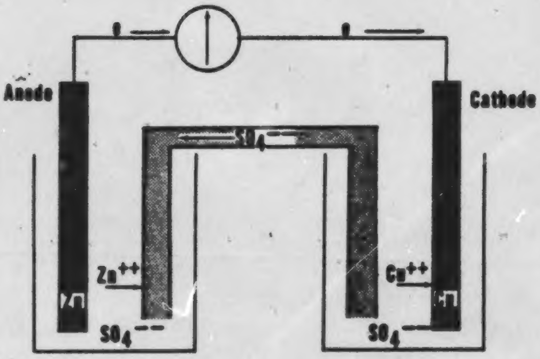
Note: Mention of Commercial Products and Manufacturers Does Not Imply Endorsement by the Environmental Protection Agency.

equivalent in terms of chemical and electrical energy and generally are reversible.

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.

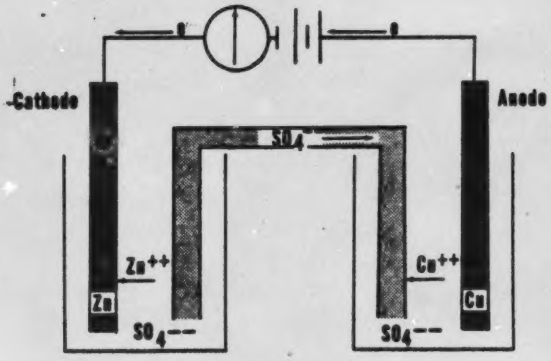
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17° C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5° C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per ° C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become so in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KCl and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches × 1/1000). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negligible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

- a Variable line voltage or low batteries in amplifier power circuits.
- b Substandard or unsteady amplifier or resistor components.
- c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.
- d Inadequately shielded electronic components.
- e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{ua}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes $DO = \frac{ua}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply USEPA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

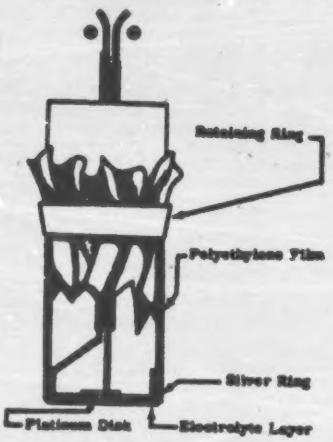

Retaining Ring
Polyethylene Film
Silver Ring
Platinum Disk
Electrolyte Layer

Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

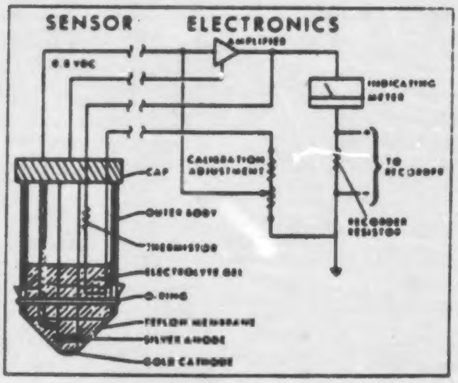

SENSOR
ELECTRONICS
AMPLIFIER
6 V DC
INDICATING METER
CALIBRATION ADJUSTMENT
TO RECORDER
CAP
OUTER BODY
RECORDER RESISTOR
THERMISTOR
ELECTROLYTE GEL
O-RING
TEFLON MEMBRANE
SILVER ANODE
GOLD CATHODE

Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor

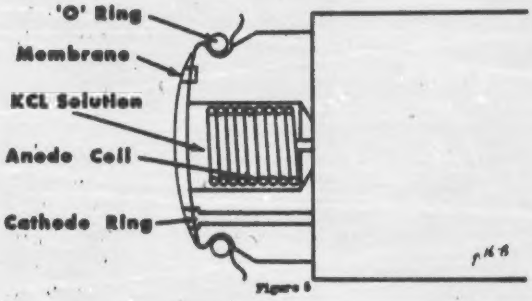

'O' Ring
Membrane
KCL Solution
Anode Coil
Cathode Ring

Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

YSI Model 54 Agitator Probe

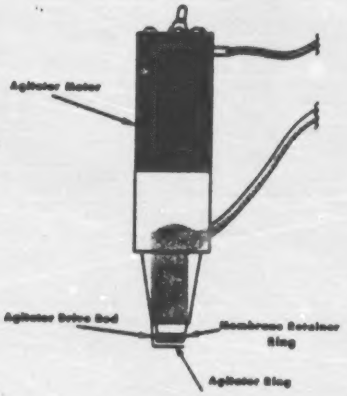

Agitator Motor
Agitator Drive Rod
Membrane Retainer Ring
Agitator Ring

Figure 6

E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

Precision Galvanic Cell Oxygen Probe

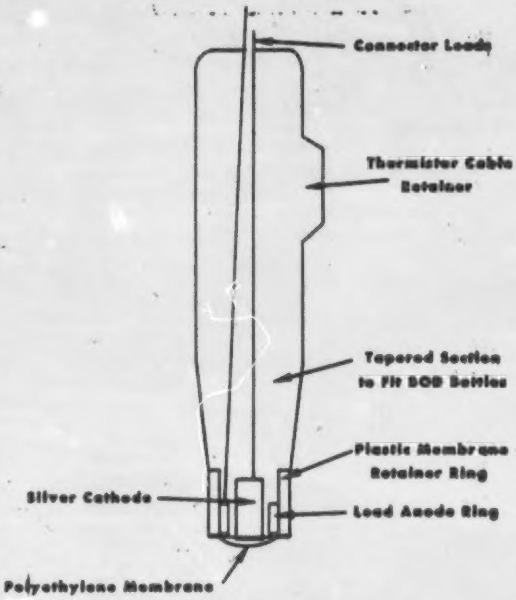

Connector Leads
Thermistor Cable Retainer
Tapered Section to Fit BOD Bottles
Plastic Membrane Retainer Ring
Silver Cathode
Lead Anode Ring
Polyethylene Membrane

Figure 7

F The Weston and Stack Model 300 DO Analyzer (3) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

ACKNOWLEDGMENTS:

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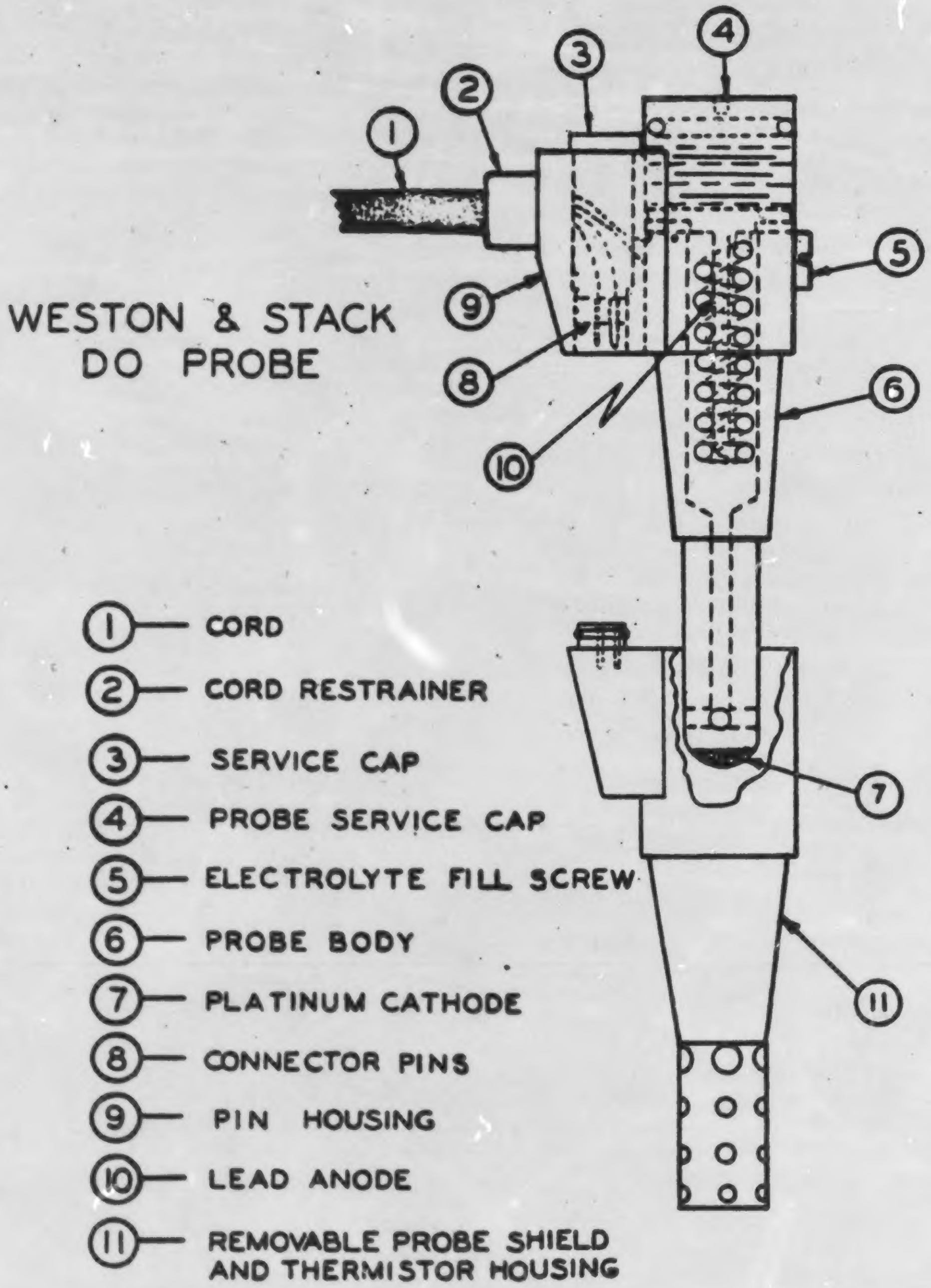
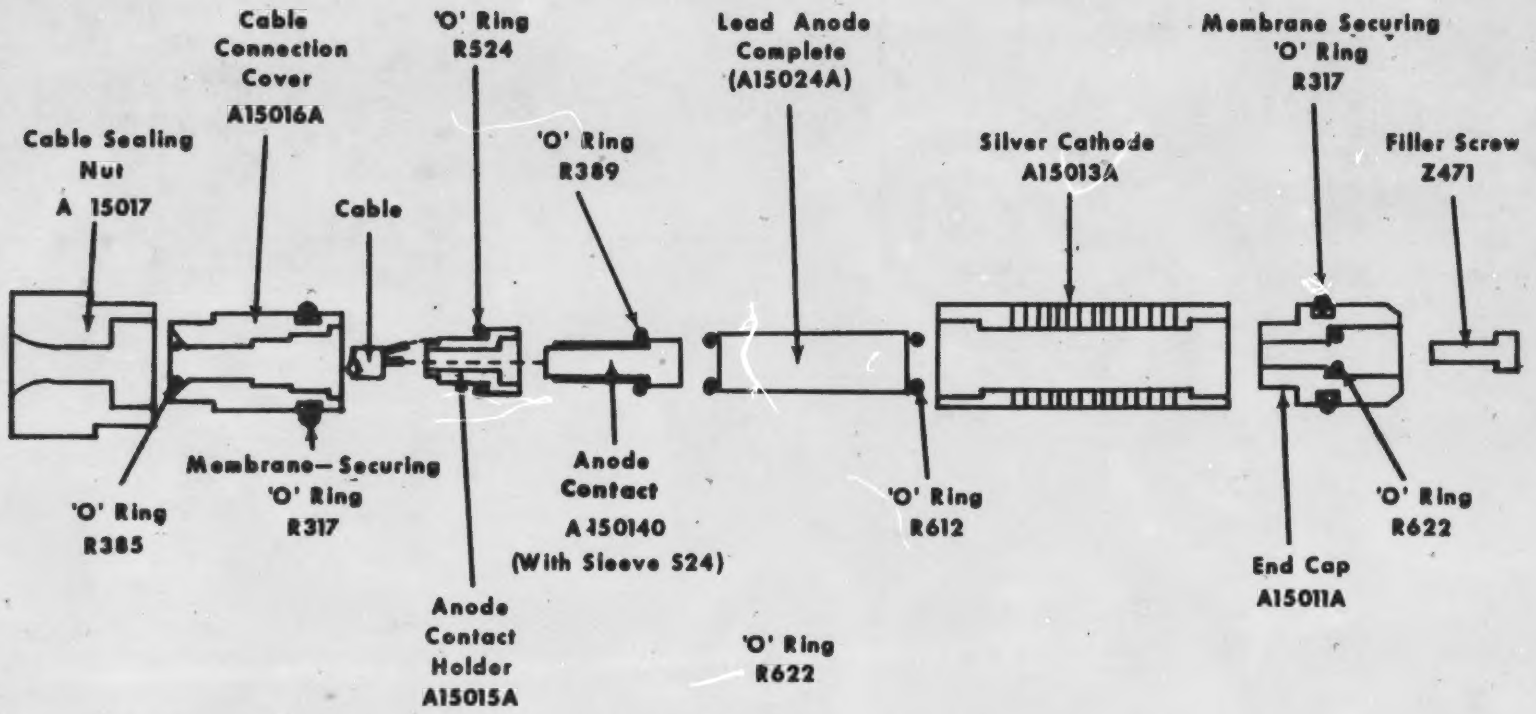

WESTON & STACK
DO PROBE
①
②
③
④
⑤
⑥
⑦
⑧
⑨
⑩
⑪
① CORD
② CORD RESTRAINER
③ SERVICE CAP
④ PROBE SERVICE CAP
⑤ ELECTROLYTE FILL SCREW
⑥ PROBE BODY
⑦ PLATINUM CATHODE
⑧ CONNECTOR PINS
⑨ PIN HOUSING
⑩ LEAD ANODE
⑪ REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS


Cable Sealing Nut A 15017
Cable Connection Cover A15016A
Cable
'O' Ring R524
'O' Ring R389
Lead Anode Complete (A15024A)
Silver Cathode A15013A
Membrane Securing 'O' Ring R317
Filler Screw Z471
'O' Ring R385
Membrane—Securing 'O' Ring R317
Anode Contact A 150140 (With Sleeve S24)
Anode Contact Holder A15015A
'O' Ring R612
'O' Ring R622
End Cap A15011A
'O' Ring R622

Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T22.

Figure 9

TABLE 1

CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp.	Temp. Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KCl KOH N/2	pol*	polyeth	no	no		Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCl gel	pol	teflon	yes	yes	yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no*	yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes	yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv**	polyeth	no	no	yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes	yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes	yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes	no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes	yes	field bottle & agitator probe

*Pol - Polarographic (or amperometric)

**Galv - Galvanic (or voltametric)

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This outline was prepared by F. J. Ludzack, former Chemist, National Training Center, MOTD, OWPO, USEPA, Cincinnati, OH 45268 and Nate Malof, Chemist, USEPA, OWPO, National Field Investigations Center, Cincinnati, OH

Descriptors: Chemical Analysis, Dissolved Oxygen, Dissolved Oxygen Analyzers, Instrumentation, On-Site Tests, Water Analysis, Analysis, Wastewater, Oxygen

LABORATORY PROCEDURE FOR DISSOLVED OXYGEN
Winkler Method-Azide Modification

I APPLICABILITY

A The azide modification is used for most wastewaters and streams which contain nitrate nitrogen and not more than 1 mg of ferrous iron/l. If 1 ml 40% KF solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg ferric iron/l.

B Reducing and oxidizing materials should be absent.

C Other materials which interfere with the azide modification are: sulfite, thiosulfate, appreciable quantities of free chlorine or hypochlorite, high suspended solids, organic substances readily oxidized in a highly alkaline medium, organic substances readily oxidized by iodine in an acid medium, untreated domestic sewage, biological flocs, and color which may interfere with endpoint detection. A dissolved oxygen meter should be used when these materials are present in the sample.

II REAGENTS

Distilled water is to be used for the preparation of all solutions.

A Manganous Sulfate Solution

Dissolve 480 g $MnSO_4 \cdot 4H_2O$ (or 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$) in water and dilute to 1 liter.

B Alkaline-Iodide-Azide Solution

Dissolve 500 g sodium hydroxide (or 700 g potassium hydroxide) and 135 g sodium iodide (or 150 g potassium iodide) in water and dilute to 1 liter. To this solution add 10 g of sodium azide dissolved in 40 ml water.

C Sulfuric Acid, Conc.

The strength of this acid is 36 N.

D Starch Solution

Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a refrigerator at 10°C.

E Sodium Thiosulfate Stock Solution 0.75 N

Dissolve 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

F Sodium Thiosulfate Standard Titrant 0.0375N

Dilute 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform.

G Potassium Biiodate Solution 0.0375N

Dry about 5 g of $KH(IO_3)_2$ at 103°C for two hours and cool in a desiccator. Dissolve 4.873 g of the solid in water and dilute to 1 liter. Dilute 250 ml of this solution to 1 liter.

H Sulfuric Acid Solution 10%

Add 10 ml of conc sulfuric acid to 90 ml of water. Mix thoroughly and cool.

I Potassium Iodide Crystals

III STANDARDIZATION OF THE TITRANT

A Dissolve 1-3 g of potassium iodide in 100-150 ml of water.

B Add 10 ml of 10% sulfuric acid and mix.

C Pipet in 20 ml of the 0.0375N potassium biiodate and mix. Place in the dark for 5 minutes.

D Titrate with the 0.0375N sodium thiosulfate standard titrant to the appearance of a pale yellow color.

Mix the solution thoroughly during the titration.

E Add 1-2 ml of starch solution and mix. The solution is now blue in color.

F Continue the addition of the titrant, with thorough mixing, until the solution turns colorless.

G Record the ml of titrant used.

H Calculate the N of the sodium thiosulfate standard titrant. It will be approximately 0.0375.

$$N = \frac{(\text{ml} \times N) \text{ of the biiodate}}{\text{ml of titrant}}$$

$$= \frac{20.0 \times 0.0375}{\text{ml of titrant}}$$

$$= \frac{0.75}{\text{ml of titrant}}$$

IV PROCEDURE

A Addition of Reagents

1 Manganous sulfate and alkaline iodide-azide

To a full BOD bottle (300 ml ± 3 ml), add 2 ml manganous sulfate solution and 2 ml alkaline-iodide azide reagent with the tip of each pipette below the surface of the liquid.

2 Stopper the bottle without causing formation of an air bubble.

3 Rinse under running water.

4 Mix well by inverting 4-5 times.

5 Allow the precipitate to settle until at least 200 ml of clear supernate have been produced.

6 Repeat steps 4 and 5.

7 Add 2 ml conc. sulfuric acid with the tip of the pipette above the surface of the liquid.

8 Stopper the bottle without causing formation of an air bubble.

9 Rinse under running water.

10 Mix by inverting several times to dissolve the precipitate.

11 Pour contents of bottle into a wide-mouth 500 ml Erlenmeyer flask.

B TITRATION

1 Titrate with 0.0375N thiosulfate to a pale yellow color.

2 Add 1-2 ml starch solution and mix.

3 Continue the addition of the titrant, with thorough mixing, until the solution turns colorless.

4 Record the ml of titrant used.

C CALCULATION

$$\text{mg DO/1} = \frac{\text{ml titrant} \times N \text{ titrant} \times 8 \times 1000}{\text{ml sample}}$$

If the N of the titrant exactly = 0.0375,

$$\text{mg DO/1} = \frac{\text{ml titrant} \times 0.0375 \times 8 \times 1000}{300}$$

$$= \text{ml titrant} \times 1$$

$$= \text{ml titrant}$$

REFERENCE
Methods for Chemical Analysis of Water & Wastes, U.S. Environmental Protection Agency, Environmental Monitoring & Support Laboratory, Cincinnati, Ohio 45268, 1974

This outline was prepared by C. R. Feldmann, Chemist, National Training and Operational VPO, Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analytical Techniques, Chemical Analysis, Dissolved Oxygen, Laboratory Tests, Oxygen, Water Analysis

DATA SHEET

ml of titrant = ____________

N of titrant = ____________

ml of sample = ____________

$$\text{mg DO/liter} = \frac{\text{ml titrant} \times \text{N titrant} \times 8 \times 1000}{\text{ml sample}}$$

$$= \frac{\quad \times \quad \times 8 \times 1000}{\quad}$$

= ____________

=

PRINCIPLES OF ABSORPTION SPECTROSCOPY

I INTRODUCTION

In any system employing principles of absorption spectroscopy, there are three basic components.

A SOURCE of Radiant Energy

B MEDIUM (Sample) which absorbs Radiant Energy

C DETECTOR to measure the Radiant Energy transmitted by the Sample

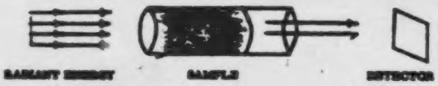


Figure 1. Basic Components of Absorption Spectroscopy System

II RADIANT ENERGY

A Wave Nature

1 The various forms of radiant energy have been arranged in a single schematic diagram referred to as the electromagnetic spectrum (see Figure 2). All of the energies which make up this spectrum may be represented graphically as waves. All waves move through space (and for most purposes air) at a constant velocity, 3×10^{10} cm/sec.

2 Three variable characteristics of individual waves serve to differentiate each from all other waves in the spectrum.

a The Wave Length

λ - The linear distance between the crests of two adjacent waves. (Units: distance/wave.)

b The Frequency

ν - The number of waves which pass a given point in a unit of time. (Units: waves/time unit.)

c The Wave Number

$\bar{\nu}$ - The number of waves which occur in a given linear distance. (Units: waves/distance unit.)

2 It is evident that more waves of short wavelength will "fit" into a given linear distance than would waves of a greater wavelength. Thus, waves having short wavelengths will have higher wave numbers. Mathematically, wave length is the reciprocal of wave number, if the same units of linear measurement are used in each expression. Since the velocity of all waves is equal and constant, it is also apparent that a greater number of waves of short wavelength can pass a given point in a unit of time than waves having a longer wavelength.

B Particle Nature

Planck conducted certain experiments which indicated that light has a particle as well as a wave nature. Energy rays can be said to consist of particles with a definite amount of energy. These particles or packets are referred to as photons or quanta. The energy (E) of each minute packet is given by Planck's equation.

$$E = h\nu \qquad (5)$$

Where E = Radiant Energy in ergs

h = Planck's proportionality constant (6.6×10^{-27} erg sec.)

ν = Frequency in waves per second

Thus, it can be seen that the energy of a given photon is directly proportional to the frequency of the given Radiant Energy.

III ABSORPTION OF ENERGY BY ATOMS AND MOLECULES

A Absorption of energies of given frequencies by atoms and molecules can be used as a basis for their qualitative identification. Absorption spectroscopy is based on the principle that certain displacements of electrons or atoms within a molecule are permissible according to the quantum theory. When radiant energy of the same energy required to bring about this permissible change is supplied to the molecule, the change occurs and energy is absorbed.

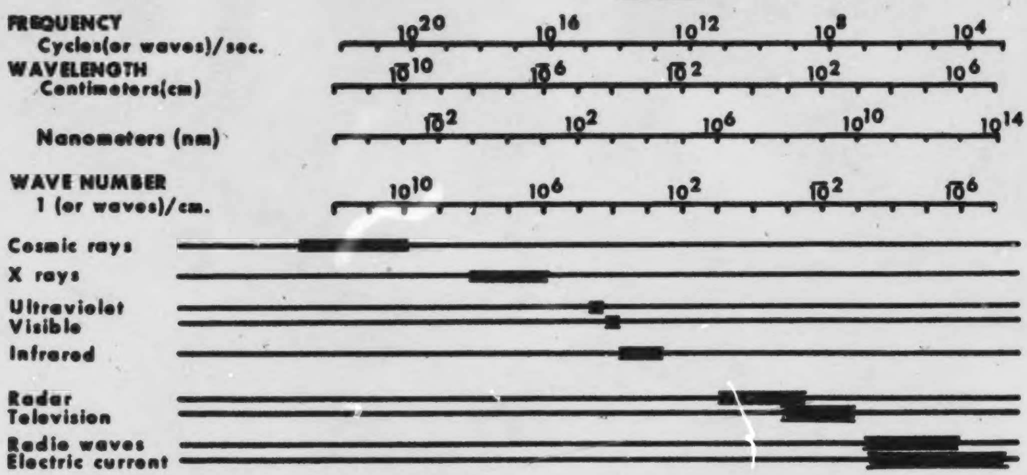
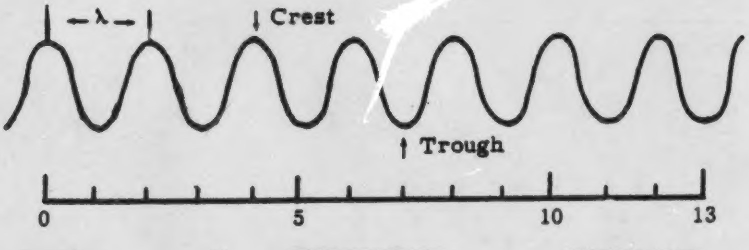


FIGURE 2



(1) WAVE LENGTH × FREQUENCY = CONSTANT

(2) $\lambda \times \nu = c$

(3) $\dfrac{\text{distance}}{\text{wave}} \times \dfrac{\text{waves}}{\text{time}} = \dfrac{\text{distance}}{\text{time}}$ = VELOCITY

(4) λ (cm/wave) × ν (waves/sec) = $C = (3 \times 10^{10}$ cm/sec)

Figure 3. Relationship of Wave Length and Frequency

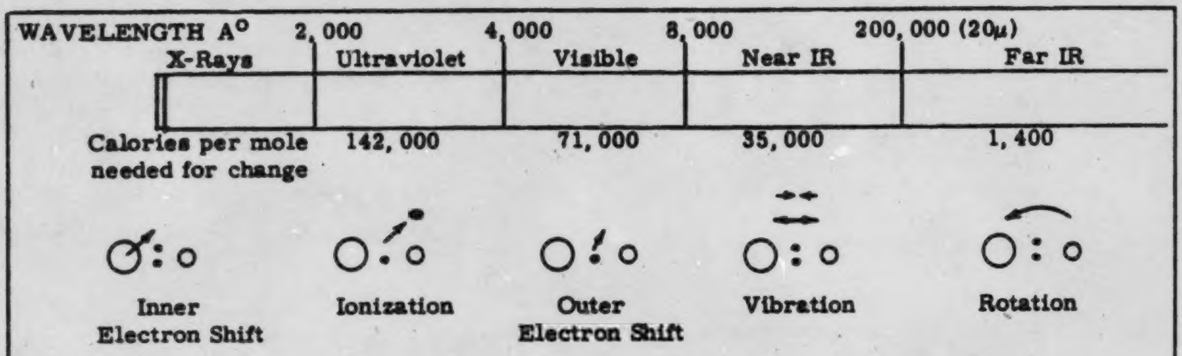


Figure 4. Electromagnetic Spectrum Showing Energy Ranges and Corresponding Electronic, Vibrational and Rotational Motions

1 Displacement of electrons is a permissible change which can occur when energy of ultraviolet and visible frequencies strikes certain atoms and molecules.

a Inner electron shift

Electrons located in the inner orbit of an atom may, when the proper frequency of radiant energy is available, shift to an orbit farther removed from the nucleus. This shift represents a change from a lower energy to a higher one. If this new position is unstable, the electron may revert to some position nearer the nucleus; the energy which was gained may then be emitted from the atom as part of its emission spectrum. The number of energy changes possible within an atom is a function of the number of electrons and the number of changes each may enter. Each possible change gives rise to a new spectral frequency. Since the frequency of radiation needed to accomplish such changes is of a high order of magnitude, the energy used is considerable in quantity.

Molecular aggregations often disintegrate in such circumstances; thus, these higher frequencies are used mainly for work with elements or very stable compounds.

b Ionization

Under a specific frequency of radiation, an electron may be physically separated from its parent atom. This process has been termed ionization. A change of energy level of this magnitude requires less energy than the inner electron shift. Such changes are characteristic of those of the rare earths, inorganic ions, transition elements and many organic compounds under frequencies within the ultraviolet range.

c Outer electron shift

The various orbital electrons in an atom may vary in the amount of energy required to shift them outwardly from the nucleus. For example, it requires less energy to shift an electron from a position more distant than it does to shift an electron outwardly from the inner orbit. Outer electron shifts occur readily in colored organic molecules for which electronic transitions are made easier by the presence of chromophore groups which participate in resonance. Thus, the excitation of the delocalized outer electrons (pi electrons) is relatively easy and requires energy in the visible range.

2 Vibration of atoms within molecules is a permissible change which can occur when energy of near infrared frequency strikes certain organic molecules.

The atoms within a molecule are held together by attractive bonding forces. Atoms within a molecule are constantly moving toward and away from other atoms, but for purposes of theory can be said to have a certain "average" position.

The change in position of an atom in relation to another atom is called vibration. The mechanics of vibration require energy; the manner and rate of vibration of the atoms depend upon frequencies of electromagnetic radiation which strikes them. Therefore, a specific part of a molecule may absorb significant quantities of certain spectral frequencies. Such absorption will be reflected in the absorption spectrum of the compound. The energy requirements for this type of energy change are of a lower order of magnitude than those above; therefore, we would expect that the frequency required would be lower and the wave length longer. Such changes occur in organic compounds under infrared radiation.

3 Rotation of molecules is a permissible change which can occur when energy of far infrared frequency strikes certain organic molecules.

A molecule rotates around its symmetrical center. The manner and rate of rotation again depends upon the energy supplied to it.

Specific spectral frequencies of electromagnetic radiation can be employed to increase the rate of rotation. The used radiation is, in effect, absorbed and reflected in the absorption spectrum.

Organic molecules utilize infrared radiation while varying their rate and manner of rotation.

B The Lambert-Beer Law provides the basis for quantitative analysis by absorption spectroscopy. It is a combination of the Bouguer (or Bouguer-Lambert) and Beer Laws.

1 Bouguer (or Bouguer-Lambert) Law

When a beam of monochromatic radiation passes through an absorbing medium, each infinitesimally small layer of the medium decreases the intensity of the beam by a constant fraction.

Mathematically:

$$\frac{-dI}{I} = k\ db \qquad (6)$$

On integration and converting base e to base 10 logarithms,

$$\log \frac{I_0}{I} = A = Kb \qquad (7)$$

$-dI$ = increment by which incident monochromatic radiation is decreased (or absorbed) by the medium.

I = intensity of the radiation emerging from the absorbing medium.

k = proportionality constant whose value depends on the wave length and the nature of the medium; i.e., the solvent used if the absorbing medium is a solution, and the temperature.

db = increment thickness of the absorbing medium.

63

I_o = radiation entering the medium.

$\log \frac{I_o}{I}$ = A = absorbance (optical density)

K = 2.303 k

b = length of radiation passing through the medium (i.e., the width of the cell, generally express in cm.)

2 Beer's Law

Each molecule of an absorbing medium absorbs the same fraction of radiation incident upon it regardless of concentration.

Mathematically:

$$\frac{-dI}{I} = k' \; dc \qquad (8)$$

On integration and converting base e to base 10 logarithms,

$$\log \frac{I_o}{I} = K' c \qquad (9)$$

k' = a proportionality constant whose value is governed by the same factors which determine the value of k.

dc = increment concentration of the absorbing medium.

K' = 2.303 k'

c = concentration of the absorbing medium (in the case of a solution c is generally expressed in moles/liter.)

3 Lambert-Beer Law

$$A = \log \frac{I_o}{D} = e \; b \; c \qquad (10)$$

e = a constant obtained by combining K plus K'. When b is expressed in cm and c in moles/liter, e is called the molar absorptivity.

4 The term transmittance is sometimes used to express how much radiation has been absorbed by a medium.

$$\text{Transmittance (T)} = \frac{I}{I_o} \qquad (11)$$

$$\text{\% Transmittance (\%T)} = \frac{I}{I_o} 100 \qquad (12)$$

The relationship between absorbance and transmittance is given by the expression:

$$A = \log \frac{I}{T}$$

5 The application of the Lambert-Beer Law to a problem involving quantitative analysis is made by the use of a calibration curve (or graph). See Figure 5.

Several standard solutions containing known concentrations of the material under analysis are "read" in the spectrophotometer. Figure 5 is prepared by graphing concentrations vs. corresponding absorbance readings.

If a straight line is obtained, the material is said to follow Beer's Law in the concentration range involved. The absorbance of the sample is then "read" and the corresponding concentration obtained from the calibration curve.

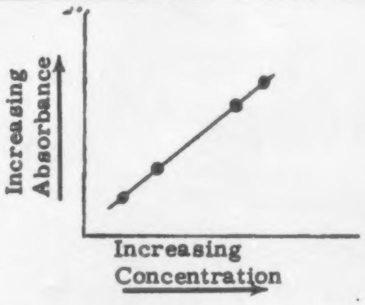


Figure 5.

ACKNOWLEDGMENT:

This outline contains certain portions from a previous outline by Betty Ann Punghorst, former Chemist, National Training Center.

RANGE	SOURCE OF RADIANT ENERGY	ABSORPTION BY SAMPLE		DETECTION OF RADIANT ENERGY TRANSMITTED
		CHEMICAL NATURE OF SAMPLE	TYPE OF SAMPLE CELL USED	
Ultraviolet	Hydrogen Arc	Inorganic ions and Organic Molecules	Quartz Fluorite	Photoelectric Cells Photographic Plates
Visible	Incandescent Tungsten Bulb	Colored Inorganic and Organic Molecules	Glass	Eye Photographic Plates Photoelectric Cells
Infrared	Nernst Glower Globar Lamp	Organic Molecules	Sodium Chloride or Potassium Bromide	Thermocouple

REFERENCES

1 Delahay, Paul. Instrumental Analysis. The MacMillan Co., New York. 1957.

2 Mellon, M. G. Analytical Absorption Spectroscopy. John Wiley & Sons, Inc., 1950.

3 Willard, Merritt & Dean. Instrumental Methods of Analysis. D. van Nostrand Co., Inc., 1958.

4 Dyer. Applications of Absorption Spectroscopy of Organic Compounds Prentice-Hall, Inc. 1965.

This outline was prepared by C. R. Feldmann, National Training Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Water Tests, Spectroscopy, Spectrophotometry

USE OF A SPECTROPHOTOMETER

I SCOPE AND APPLICATION

A Colorimetry

Many water quality tests depend on a treating a series of calibration standard solutions which contain known concentrations of a constituent of interest, and also the sample(s) with reagents to produce a colored solution. The greater the concentration of the constituent, the more intense will be the resulting color. A spectrophotometer is used to measure the amount of light of appropriate wavelength which is absorbed by equal "thicknesses" of the solutions. Results from the standards are used to construct a calibration (standard) curve. Then the absorbance value for the sample is located on the curve to determine the corresponding concentration.

B Lambert Beer Law

States the applicable relationships:

$$A = e\,b\,c$$

1 A = absorbance

2 e = molar absorptivity

3 b = light path in cm

4 c = concentration in moles/liter

II APPARATUS

A Requirements

Are given as part of the method write-up

1 The applicable wavelength is specified. The unit used is nanometers (nm).

2 The light path (cell dimension) is often open-ended, e.g., "one cm or longer." One must know the light path length in the available spectrophotometer, because it is inversely related to the usable concentrations in the test. (Longer path lengths detect lower concentrations).

3 NOTE: For National Pollutant Discharge Elimination System (permit), or for Drinking Water Regulations test requirements, check with the issuing/report agency before using light paths (cells) that differ from the length specified in the approved method. If you have permission to use an alternate path length, concentrations for the test can be adjusted accordingly. These adjustments are discussed in IV and in VII (below).

III PREPARATION OF THE SPECTROPHOTOMETER

A Phototube/Filter

1 May have to choose a phototube for use above or below a particular wavelength.

2 A filter may be required.

3 If the available instrument involves a choice, check that the phototube (and filter, if applicable,) required for the wavelength to be used is in the instrument.

4 Always handle and wipe off the phototube and/or filter with tissue to avoid leaving fingerprints.

B Cell compartment

1 This area must be kept clean and dry at all times.

C Cells

1 A set must "match" each other in optical properties. To check this, use the same solution at the same wavelength, and verify that the absorbance value is the same for each cell.

2 Alternatively, a single cell can be used if it is thoroughly rinsed after each reading.

3 Instrument cells should be free of scratches and scrupulously clean.

  a Use detergents, organic solvents or 1:1 nitric acid-water.

  b Caustic cleaning compounds might etch the cells.

  c Dichromate solutions are not recommended because of adsorption possibilities.

  d Rinse with tap, then distilled water.

  e A final rinsing and drying with alcohol or acetone before storage is a preferred practice.

D Warm-Up

1 Plug in the power cord.

2 Turn the power switch on and give it an additional half-turn to keep the needle from "pegging."

3 Wait to use until the recommended warm-up time has passed. Anywhere from 10 to 30 minutes may be required.

4 If the instrument drifts during zeroing, allow a longer time.

E Wavelength Alignment

An excellent point is the known, maximum absorption for a dilute solution of potassium permanganate which has a dual peak at 526 nm and 546 nm. Use 2 matched cells for the following steps:

1 Prepare a dilute solution of potassium permanganate (about 10 mg/l).

2 Follow the steps in VI A, Zeroing Operation, using a wavelength of 510 nm, and distilled water as a "reagent blank." Keep the water in the cell during this entire procedure.

3 Rinse the matched cell two times with tap water, then two times with the permanganate solution.

4 Fill the cell three-fourths full with the permanganate solution. Keep the permanganate solution in this cell during this entire procedure.

5 Thoroughly wipe the cell with a tissue. Hold the cell by the top edges.

6 Open the cover and gently insert the cell, aligning it to the ridge as before.

7 Close the cover.

8 Record the wavelength and the absorbance reading on a sheet of paper.

9 Remove the cell of permanganate solution and close the cover.

10 Set the wavelength control at the next graduation (+ 5nm).

11 If the needle is not at infinite (symbol ∞) absorbance, use the left knob to re-set it.

12 Insert the cell containing distilled water using the techniques noted in 5, 6 and 7 above.

13 If necessary, use the right knob to re-set the needle at zero absorbance.

14 Remove the cell and insert the cell of permanganate solution using the techniques noted in 5, 6 and 7 above.

15 Record the wavelength and the absorbance reading.

16 Repeat steps 9 through 15 above until absorbance readings are recorded at 5 nm increments from 510 nm through 560 nm.

17 Make a graph plotting absorbance readings against wavelengths. With very good resolution, there will be two peaks - one at 525 nm and one at 545 nm. A single flat topped "peak" between these two wavelengths is acceptable.

18 If the maximum absorbances[peak(s)] occur below or above 526 nm or 546 nm, and at a number of scale units different from the stated instrument accuracy, the wavelength control is misaligned. To compensate for this until the instrument can be serviced, add or subtract the error scale units when setting wavelengths for subsequent tests.

IV CALIBRATION STANDARDS

A Requirements

A set of calibration standards is required, with concentrations usable in the available spectrophotometer cell (light path length).

1 The method reference may provide a table of light path lengths and the corresponding applicable concentration range for calibration standards, so one can choose the range required for his instrument cell or sample concentration.

2 The method reference may give directions for preparing one range of concentrations for a given light path length. If your cell provides a different length, your concentration requirements can be easily calculated by recalling that the light path length is inversely related to concentration. Thus, if your cell is twice the given path length, you need the given concentrations divided by two.

3 The method reference may give directions for preparing only one range of concentrations for the calibration standards, and then not be specific about the associated path length. You will have to test if the range is applicable to your instrument by preparing the given concentrations, obtaining absorbance values for them and checking the results according to section VII (below).

B Preparation

The calibration standards required for spectrophotometric measurements are so dilute, that they are commonly prepared by diluting stronger solutions. These are described in general terms below. Weights and volumes involved in preparing these solutions for a specific test can be found in the method write-up.

1 Stock Solutions

a Prepare by weighing or measuring a small amount of a chemical containing the constituent of interest and dissolving it to a one liter volume.

b Common stock solutions have concentrations in the range of 0.1 to 1.0 mg/ml.

c Most are refrigerated for storage and some are further treated by adding a preservative. Many are stable up to six months.

2 Standard Solutions

a Prepare by diluting a stock solution (at room temperature). Common volumes are 10.0 or 20.0 ml of stock diluted to one liter.

b Resulting standard solutions have concentrations in the range of 1.0 to 10.0 ug/ml.

c Although some standard solutions may be stable for a period of time, it is a recommended practice to prepare them on the day of use.

3 Calibration (Working) Standard Solutions

a Prepare by diluting a standard solution. Usually a set of calibration standards is required so that resulting concentrations give five to seven results within the sensitivity limits of the instrument. Common volumes are 1 to 10 ml of standard solution diluted to 100 ml.

b Resulting solutions might have concentrations in the range of 0.01 and 1.0 ug/ml.

c A reagent blank (distilled water) should be included in the set of standards.

4 Adjusting Concentrations

a You may find it necessary to adjust preparation quantities given in the method write-up, because your cell (light path length) differs from the example.

b These adjustments are discussed in A Requirements (above), and are usually applied to the Standard (intermediate) Solution.

C Chemical Treatment

1 Most colorimetric methods require that the calibration standards (including the reagent blank) are to be treated as the sample. Thus, they are to be processed through pretreatments and through the test as if they were samples. Then any test effects on sample results will be compensated by the same effects on results obtained for the treated standards.

2 One should be aware that pH is a critical condition for most colorimetric reactions. Ordinarily, a pH adjustment is included in the test method and reagents include chemicals to control pH. Thus, the processed standards correspond to the samples regarding pH, and thus they correspond in degree of color development. If standards are processed in some other manner, they must be pH adjusted to correspond to the samples at the time of color development.

V SAMPLE DILUTIONS

A Concentration Limits

The concentration of the sample must result in an absorbance within the range of the calibration standards, i.e., accurately detectable in the light path provided by the instrument. A dilution before analysis may be required to accomplish this. It is not accurate to dilute a sample after processing in order to obtain a usable absorbance reading.

1 Record dilution volumes so a dilution factor can be calculated and applied to results.

2 An analyst often has a good estimate of the expected concentration of a sample if s/he routinely tests samples from the same source. In this case, a single dilution, if any, is usually sufficient.

3 If a sample is from an unknown source, the analyst has several choices.

a Process the sample. If the reading shows it is too concentrated, dilute it until you get a value in the usable range. This result is not accurate enough to report, but you now know how to dilute the sample to process it through the test to get usable results.

b Prepare at least a 50% dilution and analyze it plus an undiluted aliquot.

c Prepare a variety of dilutions.

d Use some other analytical method to get a rough estimate of the expected concentration.

B Final Volumes

1 Dilute to a final volume sufficient to rinse the measuring glassware and provide the test volume cited in the referenced method.

2 Save any undiluted sample.

VI PROCEDURE FOR USING A SPECTROPHOTOMETER

A Zeroing Operation

The following steps have been written for spectrophotometers used in this course. Check the manual for the available instrument for the steps applicable for your work.

1 Set the wavelength control to the setting specified for the standards you are testing. Approach the setting by beginning below the number and dialing up to it.

2 If a cell is in the holder, remove it.

3 Close the cell holder cover.

4 Turn the power switch/zero control (left) knob until the needle reads infinite (symbol ∞) absorbance (on the lower scale).

5 Rinse a cell two times with tap water, two times with distilled water, then two times with the reagent blank solution.

6 Fill the cell about three-fourths full with reagent blank solution.

7 Thoroughly wipe the outside of the cell with a tissue to remove fingerprints and any spilled solution. Hold the cell by the top edges.

8 Open the cell holder cover and gently slide the cell down into the sample holder.

9 Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder.

10 Close the cover and turn the light control (right) knob until the needle reads zero absorbance (on the lower scale).

11 Record an absorbance of zero for this zero concentration solution on a data sheet. (See next page).

12 Slowly remove the cell and close the cover. (No solution should spill inside the instrument). Keep the solution in the cell.

13 The needle should return to the infinite absorbance setting. It it does not:

  a Reset the needle to the ∞ absorbance mark using the power switch/zero (left) control knob.

  b Re-test the reagent blank solution using steps 7 through 12 above.

  c If the needle does not return to the ∞ absorbance mark, another setting as noted in a. and b. is required. Additional warm-up time may be necessary before these settings can be made.

B Reading Absorbances

Using a single cell in the spectrophotometers used in this course

1 Discard any solution in the cell.

2 Rinse the cell two times with tap water, and two times with distilled water. Then rinse it two times with the lowest concentration standard remaining to be tested, or with processed sample.

3 Fill the cell about three-fourths full with the same standard or sample.

4 Use a tissue to remove any fingerprints from the cell and any droplets on the outside. Hold the cell by the top edges.

5 Open the cell holder cover and gently slide the cell down into the sample holder.

6 Slowly rotate the cell until the white vertical line on the cell is in line with the ridge on the edge of the sample holder.

7 Close the cover.

8 Record the concentration of the standard and its absorbance on a data sheet. (For a sample, record its identification code and its absorbance on the data sheet).

DATA SHEET	
Concentration mg/liter	Absorbance
0.00	
SAMPLE	
SAMPLE	

9 Repeat steps 1 through 8 (above) for each standard and sample to be tested. If a large number of measurements are to be made, check the instrument calibration every fifth reading.

a Use another aliquot of a solution already tested to see if the same reading is obtained. If not, repeat the zeroing operation in A (above).

b Alternatively, you can use the blank, if supply permits, and repeat the zeroing operation in A. (above).

10 When all the readings have been obtained, discard any solution remaining in the cell and rinse the cell with tap water. Clean the cell more thoroughly, (III C. 3), as soon as possible.

11 If no other tests are to be done, turn off the instrument, pull out the plug and replace any protective covering.

VII CHECKING RESULTS

A Readings Greater Than 0.70

On our instrument, these are considered to be inaccurate. Check the manual for your instrument or check the scale divisions to determine the limit for other models.

1 Do not use readings greater than 0.70 to develop a calibration curve.

2 From five to eight points (counting zero) are recommended for constructing a calibration curve. If you have fewer than five usable values, you should not draw a curve.

3 To prevent excessively high values in future tests, decrease the cell path length, if possible, by using an adapter and smaller cell.

4 If you cannot decrease the cell path length, you can at least obtain enough values to construct a curve. Prepare standards with five to eight concentrations ranging from zero to the concentration of the standard having an absorbance nearest to 0.70. This gives you more values for a curve, but it reduces the applicable range of the test. Usually the sample can be diluted before testing so the result will fit on the standard curve.

B Highest Reading is Less Than 0.6

1 Increase the cell path length by using a larger cell. A higher reading results.

2 Prepare a different set of standards with greater concentrations.

11-6

VIII CONSTRUCTING A CALIBRATION CURVE

If you have from five to eight usable absorbance values, you can construct a concentration curve.

A Graph Paper

Should be divided into squares of equal size in both directions

B Concentration Axis

1 Labeling

The longer side should be labeled at equal intervals with the concentrations of the calibration standards marked from 0.0 to at least the highest concentration recorded for the standards on the data sheet.

2 Units

a It is most convenient to express these concentrations in the units to be reported. Otherwise, a unit-conversion factor would have to be applied to obtain final, reportable values every time you use the curve.

b Example: If you dilute a standard solution to make 100.0 ml volumes of calibration standards, you have a choice in expressing the resulting concentrations. You can use weight/100 ml, or you can calculate weight/1 liter. If you are to report results as weight/liter, but you construct your curve using weight/100 ml, you will have to multiply every sample result from the curve by $\frac{1000}{100}$ or 10 to obtain the reportable value. It is much easier to convert the original calibration standard concentrations to the desired units and to use these as labels on the graph.

C Absorbance Axis

1 Labeling

The shorter side should be labeled at equal intervals with absorbance numbers marked from 0.00 to at least 0.70 absorbance units.

D Plotting the Curve

1 Use the absorbances recorded for each standard concentration to plot points for the curve.

2 The points should fall in a reasonably straight line.

3 Use a straight-edge to draw a line of best fit through the points. If the points do not all fall on the line, an acceptable result is an equal number of points falling closely above, as well as below the line. Experience provides a basis for judging acceptability.

4 It is not permissable to extrapolate the curve.

IX USING THE CALIBRATION CURVE

A Finding concentration of the sample

1 Use the absorbance value(s) recorded for the sample(s), and the calibration curve to find the concentration(s). If the concentration units differ from those required for reporting results, apply a unit conversion factor (VIIIB. 2).

2 If more than one dilution of a sample was tested, use the result that falls nearest the middle of the curve.

B If a sample was diluted, calculate the dilution factor and apply it to the concentration you find for the sample from the calibration curve.

1 Dilution Factor=

$$\frac{\text{final dilution volume}}{\text{ml sample used in dilution}}$$

2 Example: You diluted 10 ml sample to 50 ml. The concentration found by using a calibration curve was 0.5 mg/liter.

Then·

$$\text{constituent, mg/l} = \frac{50\text{ml}}{10\text{ ml}} \times \frac{0.5\text{ mg}}{\text{liter}}$$

$$= 2.5\text{ mg/liter}$$

This outline was prepared by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Analytical Techniques, Chemical Analysis, Colorimetry, Laboratory Tests, Spectrophotometry

ATOMIC ABSORPTION SPECTROPHOTOMETRY

I INTRODUCTION

Atomic absorption spectroscopy has been well known to physicists and astronomers for more than 100 years. In 1850, Kirchoff took light from the sun and collimated it with a lens through the flame of an ordinary laboratory burner, and then passed the light through a prism which dispersed it into the characteristic visible spectrum with which we are all familiar. He then took a platinum spoon containing a sodium salt and introduced it into the flame. He observed that the yellow light that was present in the spectrum disappeared and in its place appeared the characteristic resonance lines of sodium. Since then astronomers have used the technique to detect and measure the concentration of metals in the vapors of stars. In 1953, Walsh(1) recognized its potential advantage over emission spectroscopy for trace metal analysis. He designed and built an analytically useful atomic absorption instrument. Shortly thereafter the advantages of atomic absorption instrumentation were recognized in the United States.

II THEORY

The basis of the method is the measurement of the light absorbed at the wavelength of a resonance line by the unexcited atoms of the element. Elements not themselves excited to emission by a flame may be determined in a flame by absorption provided that the atomic state is capable of existence.

At the temperature of a normal air-acetylene flame (2100°C) only about one per cent of all atoms is excited to emission in a flame; therefore absorption due to a transition from the ground electronic state to a higher energy level is virtually an absolute measure of the number of atoms in the flame, and the concentration of the element in the sample. Electrons will absorb energy at the same characteristic wavelength at which they emit energy. This is the principle upon which the technique of atomic absorption spectroscopy is based.

The advantages of atomic absorption spectroscopy as compared to emission spectroscopy are: (1) that atomic absorption is independent of the excitation potential of the transition involved and (2) that it is less subject to temperature variation and interference from extraneous radiation and interference from extraneous radiation or energy exchange between atoms.

Atomic absorption analytical apparatus (Figure 1) consists of a suitable source of light emitting the line spectrum of an element, a device for vaporizing the sample, a means of line isolation (monochomator or filter) and photoelectric detecting and measuring equipment.

If the detector is placed to receive only the resonance line of the element from the light source, measurement can be made of the absorption of resonance-line radiation on its passage through the vaporized sample. The magnitude of this absorption gives a measure of the concentration of free ground-state atoms of the element in the vapor and when referred to a calibration curve, provides a means of determining the concentration of the element in the sample.

III INSTRUMENTATION

The general arrangement of an absorption flame photometer is no different from an emission flame photometer except for the addition of a light source. An aerosol is introduced into a flame which is placed on the optical axis between the entrance slit of the monochromator and the monochromatic light source. Energy of the wavelength absorbed by the sample is provided by a source lamp whose emitting cathode is made of that element. This energy is passed through the flame and then through a dispersing device. A detector measures the absorbed and unabsorbed exciting radiation.

A Light Source

For the more volatile elements such as the alkali metals, mercury and thallium,

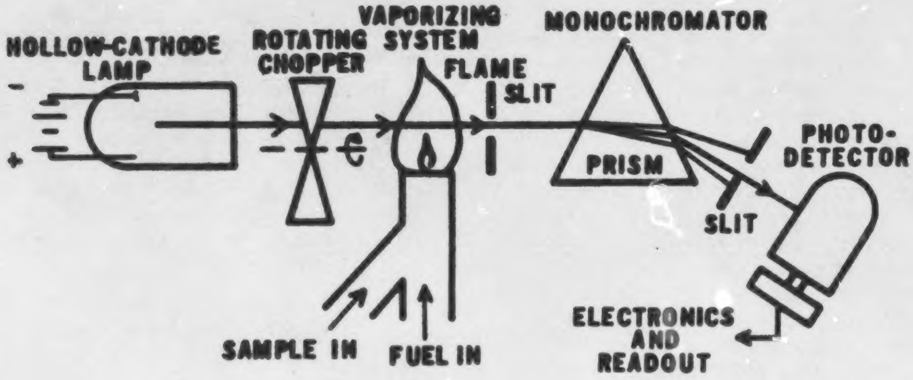

HOLLOW-CATHODE LAMP
ROTATING CHOPPER
VAPORIZING SYSTEM
FLAME
SLIT
MONOCHROMATOR
PRISM
PHOTO-DETECTOR
SLIT
ELECTRONICS AND READOUT
SAMPLE IN
FUEL IN
-
+

FIGURE I

the most convenient source is the spectral vapor lamp, which consists of a closed glass or silica tube, into which are sealed oxide-coated electrodes, containing one of the rare gases and some of the appropriate metals. For line sources of the less-volatile elements, hollow-cathode discharge tubes have been found the most satisfactory. These consist of an anode and hallow cylindrical cathode (either composed of or lined with the appropriate metal) mounted in a sealed glass tube containing one of the rare gases (helium or argon).

Lamps are operated at low currents to improve linearity of response and maintain narrow emission lines.

B Vaporization of Sample

1 Atomic-absorption methods have been applied almost exclusively to the analysis of solutions and for this purpose flames, fed with a fine spray of the sample solution, similar to those employed in flame photometry are used.

The burner has two principal functions to perform:

a It must introduce the sample into the flame.

b It must reduce the metal to the atomic state.

2 Burners can be classified as:

a Total-consumption

Those which introduce the sample spray directly into the flame. (Figure 2)

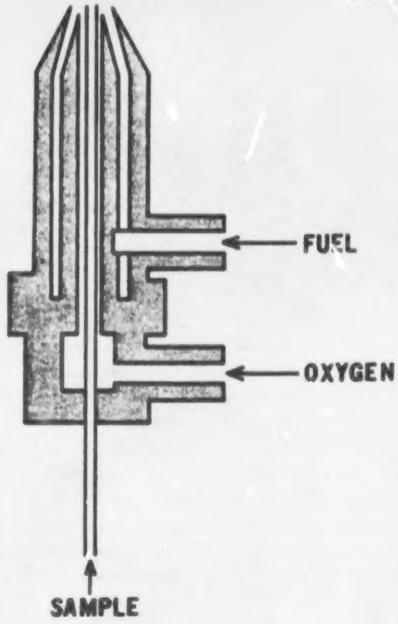

FUEL
OXYGEN
SAMPLE

FIGURE 2

b Premix

Those which introduce the spray into a condensing chamber for removing large droplets. (Figure 3)

3 Flame shape is important. The flame should have a long path length (but a narrow width, such as a fishtail flame) so that the source traverses an increased number of atoms capable of contributing to the absorption signal.

4 The effective length of the flame may be increased by multiple passages through the flame with a reflecting mirror system, or by alignment of several burners in series.

5 The flame temperature need only be high enough to dissociate molecular compounds into the free metal atoms.

Typical flame temperatures are shown in Table I.

Table I

Fuel-Oxidant	Approximate Temp., °C
Nitrous Oxide - acetylene	3000
Hydrogen - air	2100
Hydrogen - oxygen	2700 - 2800
Acetylene - oxygen	3100
Acetylene - air	2000 - 2200
Propane - oxygen	2700 - 2800
Illuminating gas - oxygen	2800
Cyanogen - oxygen	4300

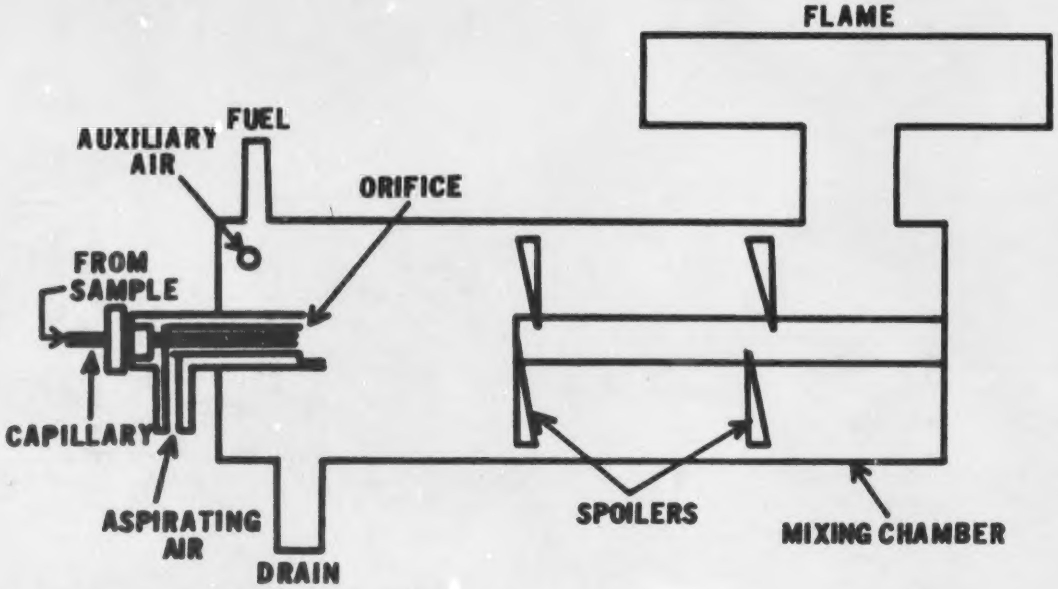


Figure 3

C Line Isolation

1 The use of a line spectrum of the element being determined, rather than a continuous spectrum, makes possible the use of monochromators of low resolving power or even filters. When a spectral lamp is used as a light source, it is only necessary to isolate the resonance line from neighboring lines of the light source or vaporized sample. The resolution of the method is implicit in the width of the emission and absorption lines.

2 To realize the full potentialities of the method, the strongest absorption line must be used. For elements with simple spectra, the resonance line arising from the lowest excited state is usually the line exhibiting strongest absorption.

3 Calibration curves depart from linearity at much lower concentrations in absorption work as compared with emission work. Curvature results partly from increased pressure broadening as the concentration of salt rises, but also depends on source characteristics, particularly self-absorption, and on the nature and homogeneity of the flame.

D Detection

1 Photo-electric detectors used in atomic absorption analysis need be no more sensitive than those used in emission analysis, since in the atomic absorption method, concentration of an element is determined by measuring the reduction in intensity of the resonance line emitted from a source of high intensity.

2 Single or double-beam circuits may be adopted for work with a single beam instrument, results are directly dependent upon source and detector stability. Both must be powered by separate power supplies. In a double-beam system small variations in the source signal are compensated automatically.

IV EVALUATION

A Sensitivity

1 For an air-acetylene flame of length 2 or 3 cm the lower limits of detection of elements having low resonance-line excitation potential (eg Na-K) are approximately equal in a single-beam atomic absorption and emission methods.

2 For elements having highly reversed resonance lines or resonance lines of high excitation potential, the atomic-absorption method has decided advantages over emission methods. Examples of elements in these categories are Zn, Mg, Fe and Mn.

3 A disadvantage of the atomic-absorption method, when compared with flame emission, is the lack of a quick and simple method of varying sensitivity to deal with solutions of widely varying element concentrations. The sensitivity of an atomic-absorption instrument is determined almost entirely by flame characteristics, notably length of light path through the flame.

4 A comparison of sensitivity obtained by emission and adsorption techniques is given in Table II.

B Precision

1 Precision of a single-beam atomic absorption instrument is primarily a function of the stability of light output from the spectral lamp. This in turn is dependent on the stability of the main supply and inherent stability of the lamp. The largest fluctuations are only ± 2 percent for the hollow cathode tube and sodium spectral vapor lamp. A double-beam instrument significantly reduces this error.

2 In common with flame-emission methods, atomic absorption is subject to "noise" from the flame and the detector. Changes in absorption caused by fluctuations in

Element	Sensitivity mg/l Flame	A. A
Aluminum	2	0.5
Antimony		0.2
Arsenic		1.0
Barium	0.3	1.0
Beryllium	25	0.05
Bismuth		0.2
Cadmium	2	0.01
Calcium	0.003	0.01
Cesium		0.05
Chromium	0.1	0.01
Cobalt		0.15
Copper	0.01	0.005
Gallium		1.0
Gold		0.1
Imdium		0.5
Iron	0.2	0.05
Lead	2	0.15
Lithium	0.002	0.005
Magnesium	0.1	0.003
Manganese	0.01	0.01
Mercury	10	0.5
Molybdenum		0.2
Nickel		0.05
Palladium		1.0
Platinum		0.5
Potassium	0.001	0.005
Rhodium		0.3
Rubidium		0.02
Selenium		1.0
Silver	0.05	0.02
Sodium	0.002	0.005
Strontium	0.01	0.02
Tellurium		0.5
Titanium		1.0
Thallium		0.2
Tin		2.0
Vanadium		0.5
Zinc	200	0.005

Table II

flame temperature are much less than those in emission because the strength of the absorption line is principally dependent on Doppler width whereas the intensity of emission from the flame is much more sensitive to temperature.

C Accuracy

This is shown by the types of interference found in flame emission and atomic absorption spectroscopy. There are three types:

1 Physical

Collision of atoms and electrons or atoms and molecules will transfer energy thus causing an enhancement or depression of analysis-line emission.

This has a large effect on flame emission analysis but has only a negligible effect on atomic absorption.

2 Radiative

Light from elements other than the one being measured pass the line isolating device (monochromator or filter). This occurs in flame emission work, for example, the interference of calcium and magnesium in sodium determinations. This interference is also encountered in atomic absorption using a D.C. system but is very small because of the large signal from the hollow-cathode tube. Radiative interference is eliminated in an A.C. system.

3 Chemical

Emission from an element in the flame is depressed by the formation of compounds, which are not dissociated at flame temperatures. This also affects absorption because the formation of temperature - stable compounds in the flame causes proportionate reduction in the population of ground-state and excited atoms.

Investigations to date suggest chemical interference is confined, almost entirely, to the alkaline-earth elements and that calcium absorption is more subject to this interference than is magnesium absorption.

Typical calibration curves are shown in Figure 5.

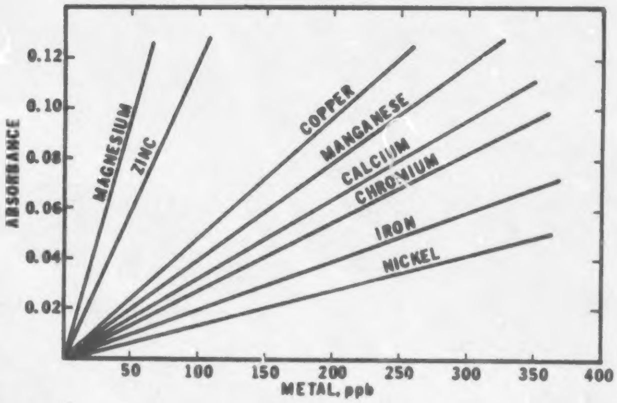


Figure 5

12-5

V REMOVAL OF INTERFERENCES AND CONCENTRATION OF SAMPLE

A Removal of Interferences

1 The methods for overcoming these interferences in atomic absorption are similar to those used in flame emission, namely, either separation of interfering ions or suppression of the interference by addition in excess of a substance that will prevent formation of compounds between interfering ions and the element being determined.

B Concentration of Sample

1 Organic separations can be used to concentrate a sample. Interferences are removed, as seen above, and also the organic solvent enhances the absorption.

2 Ion exchange has also been used successfully for concentrating samples for atomic absorption.

VI CONCLUSIONS

Atomic absorption methods are as good as or better than emission methods, for elements to which they can both be applied, in sensitivity, precision and accuracy. They can be applied to a far wider range of elements than can emission analysis. The additional cost of hollow cathode discharge tubes is compensated by the greater range of analyses and greater reliability of results.

VII INSTRUMENTS AVAILABLE

A Perkin Elmer

1 Model 303 - double beam, AC - $5,920.00

1 Model 290 - single beam, AC - $2,900.00

B Beckman attachments for existing spectrophotometers

1 Use with model D. U. and D. U. -2- single beam, DC - $2,135.00

2 Use with model D. B. - single beam, AC - $2,495.00

C Jarrell-Ash

1 Dual atomic absorption flame spectrometer- single beam, AC - $5,800.00

D E. E. L.

1 Atomic absorption spectrophotometer - single beam, AC - $2,850.00

ACKNOWLEDGEMENT

Certain portions of this outline contain training meterial from a prior outline by Nathan C. Malof.

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This outline was prepared by P. F. Hallbach, Chemist, National Training Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analytical Techniques, Atomic Absorption, Instrumental Analysis, Metals Analysis

ATOMIC ABSORPTION LABORATORY

ZINC ANALYSIS

Preparation of Standards:

A stock standard zinc solution was prepared by dissolving 1.000 grams of pure zinc metal in nitric acid and diluting to 1 liter with distilled water for a concentration of 1 ml = 1 mg Zn. Appropriate dilutions were made to provide 0, 0.1, 0.3, 0.5, 0.8, and 1.0 mg zinc/l standards.

Operating Conditions for P-E 303:

Wavelength	214
Range	UV
Slit	5
Source	10 ma, hollow cathode
Burner	P-E premix
Air	20 psi. (30 psi. on tank)
Auxiliary Air	9.0 on flowmeter
Acetylene	8 psi; 9.0 on flowmeter
Flame	clear and non-luminescent
Sample Uptake Rate	2-5 ml/min.

ATOMIC ABSORPTION LABORATORY

Work Sheet

mg Zn/1	% Absorption	Absorbance
0.1	____________	____________
0.3	____________	____________
0.5	____________	____________
0.8	____________	____________
1.0	____________	____________
unknown		

Concentration of unknown = ____________ mg Zn/1

Scale expansion = ____________

Suppression (meter response) = ____________

This outline was prepared by R. J. Lishka, Research Chemist, Bureau of Water Hygiene, EPA, Cincinnati, OH 45268.

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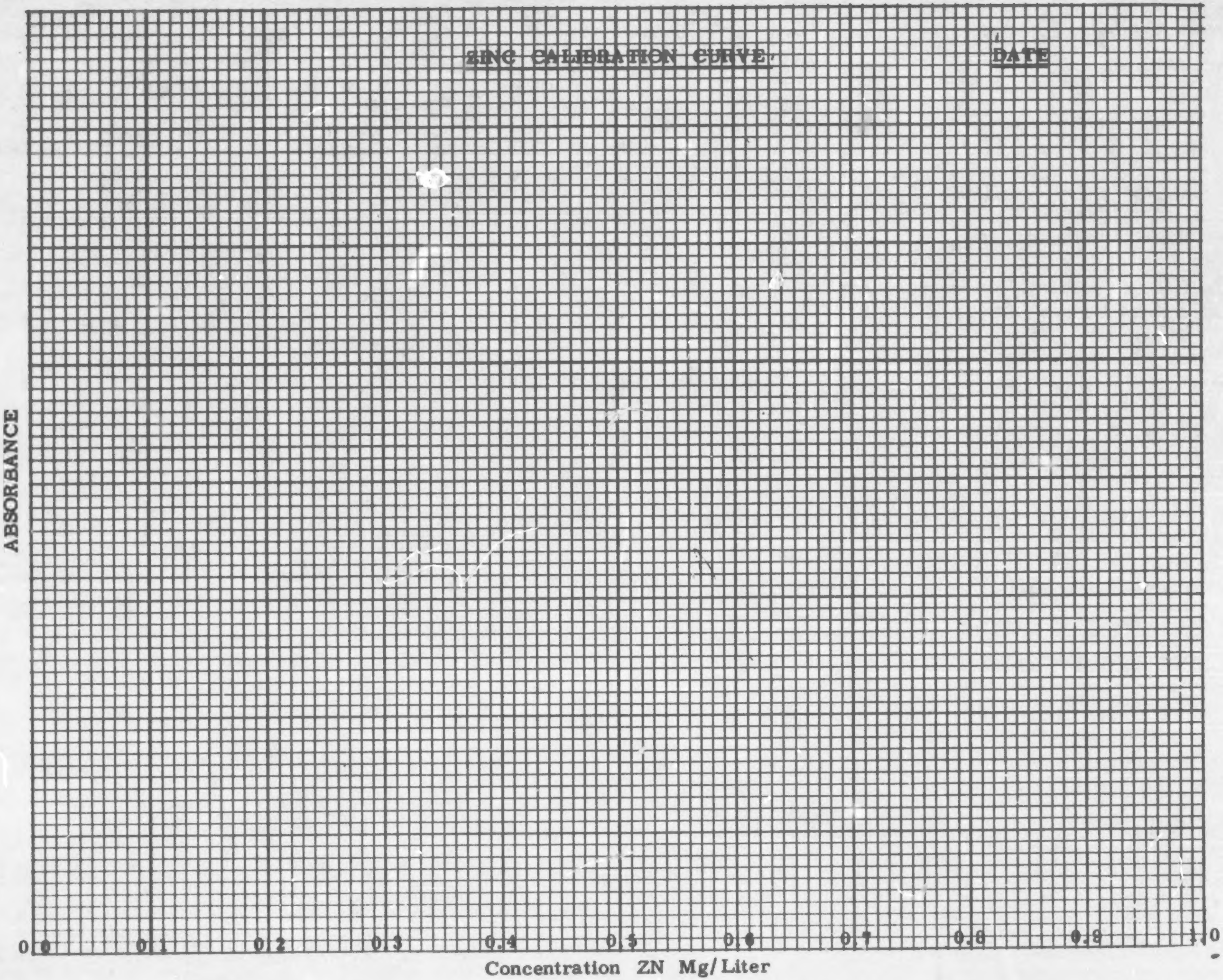

ZINC CALIBRATION CURVE
DATE
ABSORBANCE
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Concentration ZN Mg/Liter

13-3

ATOMIC ABSORPTION LABORATORY

COPPER ANALYSIS

Preparation of Standards:

A stock standard copper solution was prepared by dissolving 1.0000 gram of pure copper metal in nitric acid and diluting to 1 liter with distilled water for a concentration of 1 ml = 1 mg Cu. Appropriate dilutions were made to provide 0, 0.1, 0.3, 0.5, 0.8, and 1.0 mg copper/l standards.

Operating Conditions for P-E 303:

Wavelength	325
Range	UV
Slit	4
Source	15 ma., hollow cathode
Burner	P-E premix
Air	20 psi. (30 psi. on tank)
Auxiliary Air	9.0 on flowmeter
Acetylene	8 psi.; 9.0 on flowmeter
Flame	clear and non-luminescent
Sample Uptake Rate	2-5 ml/min.

ATOMIC ABSORPTION LABORATORY

Work Sheet

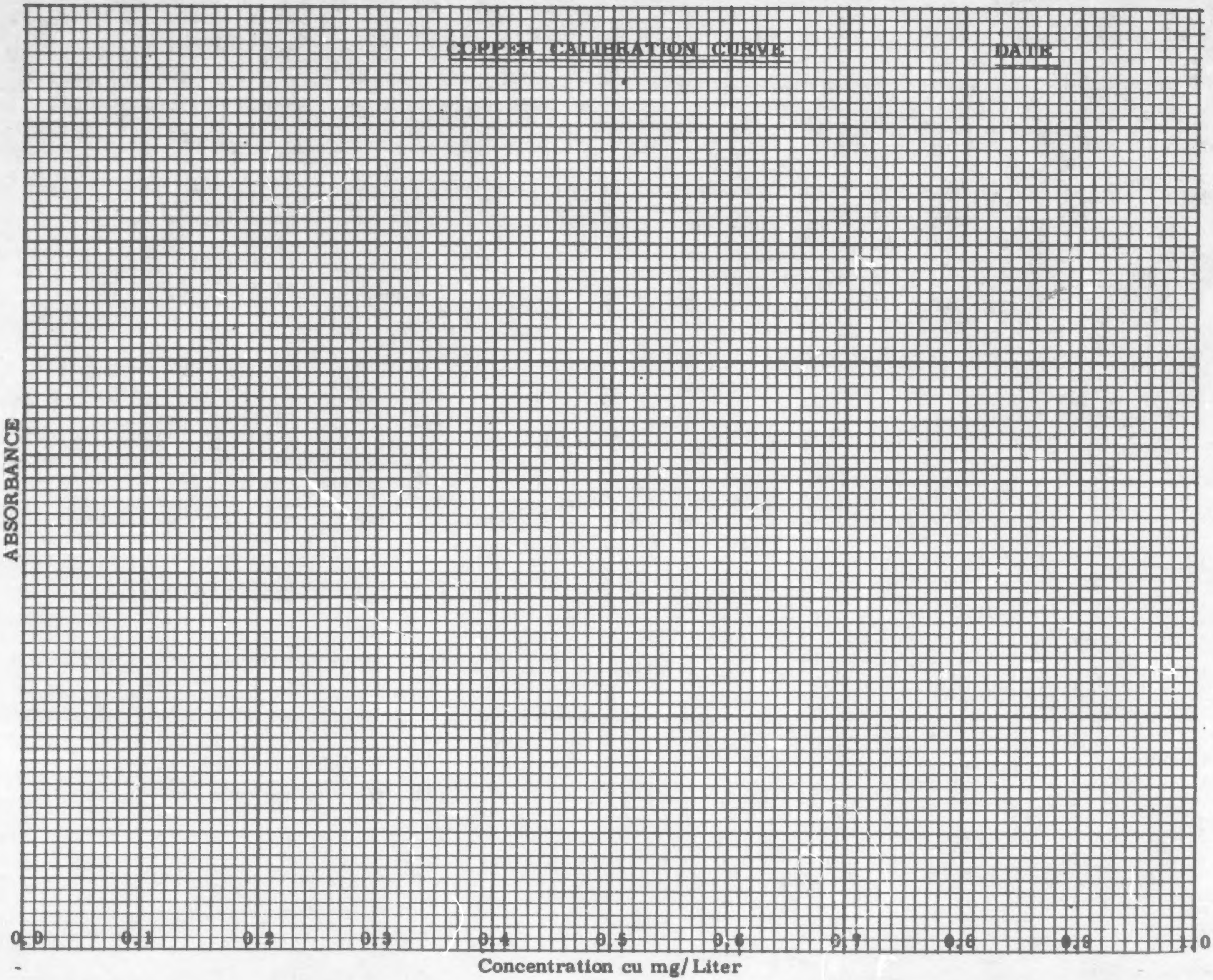
mg Cu/l	% Absorption	Absorbance
0.1	______	______
0.3	______	______
0.5	______	______
0.8	______	______
1.0	______	______
unknown	______	______

Concentration of unknown = ____________ mg Cu/l

Scale expansion = ________________

Suppression (meter response) = ________________

This outline was prepared by R. J. Lishka, Research Chemist, Bureau of Water Hygiene, EPA, Cincinnati, OH 45268.


COPPER CALIBRATION CURVE
DATE
ABSORBANCE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Concentration cu mg/Liter

14-3

TABLE III - VALUES OF ABSORBANCE FOR PER CENT ABSORPTION

To convert per cent absorption (%A) to absorbance, find the per cent absorption to the nearest whole digit in the left-hand column; read across to the column located under the tenth of a per cent desired, and read the value of absorbance. The value of absorbance corresponding to 26.8% absorption is thus 0.1355.

%A	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0.0	.0000	.0004	.0009	.0013	.0017	.0022	.0026	.0031	.0035	.0039
1.0	.0044	.0048	.0052	.0057	.0061	.0066	.0070	.0074	.0079	.0083
2.0	.0088	.0092	.0097	.0101	.0106	.0110	.0114	.0119	.0123	.0128
3.0	.0132	.0137	.0141	.0146	.0150	.0155	.0159	.0164	.0168	.0173
4.0	.0177	.0182	.0186	.0191	.0195	.0200	.0205	.0209	.0214	.0218
5.0	.0223	.0227	.0232	.0236	.0241	.0246	.0250	.0255	.0259	.0264
6.0	.0269	.0273	.0278	.0283	.0287	.0292	.0297	.0301	.0306	.0311
7.0	.0315	.0320	.0325	.0329	.0334	.0339	.0343	.0348	.0353	.0357
8.0	.0362	.0367	.0372	.0376	.0381	.0386	.0391	.0395	.0400	.0405
9.0	.0410	.0414	.0419	.0424	.0429	.0434	.0438	.0443	.0448	.0453
10.0	.0458	.0462	.0467	.0472	.0477	.0482	.0487	.0491	.0496	.0501
11.0	.0506	.0511	.0516	.0521	.0526	.0531	.0535	.0540	.0545	.0550
12.0	.0555	.0560	.0565	.0570	.0575	.0580	.0585	.0590	.0595	.0600
13.0	.0605	.0610	.0615	.0620	.0625	.0630	.0635	.0640	.0645	.0650
14.0	.0655	.0660	.0665	.0670	.0675	.0680	.0685	.0691	.0696	.0701
15.0	.0706	.0711	.0716	.0721	.0726	.0731	.0737	.0742	.0747	.0752
16.0	.0757	.0762	.0768	.0773	.0778	.0783	.0788	.0794	.0799	.0804
17.0	.0809	.0814	.0820	.0825	.0830	.0835	.0841	.0846	.0851	.0857
18.0	.0862	.0867	.0872	.0878	.0883	.0888	.0894	.0899	.0904	.0910
19.0	.0915	.0921	.0926	.0931	.0937	.0942	.0947	.0953	.0958	.0964
20.0	.0969	.0975	.0980	.0985	.0991	.0996	.1002	.1007	.1013	.1018
21.0	.1024	.1029	.1035	.1040	.1046	.1051	.1057	.1062	.1068	.1073
22.0	.1079	.1085	.1090	.1096	.1101	.1107	.1113	.1118	.1124	.1129
23.0	.1135	.1141	.1146	.1152	.1158	.1163	.1169	.1175	.1180	.1186
24.0	.1192	.1198	.1203	.1209	.1215	.1221	.1226	.1232	.1238	.1244
25.0	.1249	.1255	.1261	.1267	.1273	.1278	.1284	.1290	.1296	.1302
26.0	.1308	.1314	.1319	.1325	.1331	.1337	.1343	.1349	.1355	.1361
27.0	.1367	.1373	.1379	.1385	.1391	.1397	.1403	.1409	.1415	.1421
28.0	.1427	.1433	.1439	.1445	.1451	.1457	.1463	.1469	.1475	.1481
29.0	.1487	.1494	.1500	.1506	.1512	.1518	.1524	.1530	.1537	.1543
30.0	.1549	.1555	.1561	.1568	.1574	.1580	.1586	.1593	.1599	.1605
31.0	.1612	.1618	.1624	.1630	.1637	.1643	.1649	.1656	.1662	.1669
32.0	.1675	.1681	.1688	.1694	.1701	.1707	.1713	.1720	.1726	.1733
33.0	.1739	.1746	.1752	.1759	.1765	.1772	.1778	.1785	.1791	.1798
34.0	.1805	.1811	.1818	.1824	.1831	.1838	.1844	.1851	.1858	.1864
35.0	.1871	.1878	.1894	.1891	.1898	.1904	.1911	.1918	.1925	.1931
36.0	.1938	.1945	.1952	.1959	.1965	.1972	.1979	.1986	.1993	.2000
37.0	.2007	.2013	.2020	.2027	.2034	.2041	.2048	.2055	.2062	.2069
38.0	.2076	.2083	.2090	.2097	.2104	.2111	.2118	.2125	.2132	.2140
39.0	.2147	.2154	.2161	.2168	.2175	.2182	.2190	.2197	.2204	.2211
40.0	.2218	.2226	.2233	.2240	.2248	.2255	.2262	.2269	.2277	.2284
41.0	.2291	.2299	.2306	.2314	.2321	.2328	.2336	.2343	.2351	.2358

TABLE III CONTINUED

% A	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
42.0	.2366	.2373	.2381	.2388	.2396	.2403	.2411	.2418	.2426	.2434
43.0	.2441	.2449	.2457	.2464	.2472	.2480	.2487	.2495	.2503	.2510
44.0	.2518	.2526	.2534	.2541	.2549	.2557	.2565	.2573	.2581	.2588
45.0	.2596	.2604	.2612	.2620	.2628	.2636	.2644	.2652	.2660	.2668
46.0	.2676	.2684	.2692	.2700	.2708	.2716	.2725	.2733	.2741	.2749
47.0	.2757	.2765	.2774	.2782	.2790	.2798	.2807	.2815	.2823	.2832
48.0	.2840	.2848	.2857	.2865	.2874	.2882	.2890	.2899	.2907	.2916
49.0	.2924	.2933	.2941	.2950	.2958	.2967	.2976	.2984	.2993	.3002
50.0	.3010	.3019	.3028	.3036	.3045	.3054	.3063	.3072	.3080	.3089
51.0	.3098	.3107	.3116	.3125	.3134	.3143	.3152	.3161	.3170	.3179
52.0	.3188	.3197	.3206	.3215	.3224	.3233	.3242	.3251	.3261	.3270
53.0	.3279	.3288	.3298	.3307	.3316	.3325	.3335	.3344	.3354	.3363
54.0	.3372	.3382	.3391	.3401	.3410	.3420	.3429	.3439	.3449	.3458
55.0	.3468	.3478	.3487	.3497	.3507	.3516	.3526	.3536	.3546	.3556
56.0	.3565	.3575	.3585	.3595	.3605	.3615	.3625	.3635	.3645	.3655
57.0	.3665	.3675	.3686	.3696	.3706	.3716	.3726	.3737	.3747	.3757
58.0	.3768	.3778	.3788	.3799	.3809	.3820	.3830	.3840	.3851	.3862
59.0	.3872	.3883	.3893	.3904	.3915	.3925	.3936	.3947	.3958	.3969
60.0	.3979	.3990	.4001	.4012	.4023	.4034	.4045	.4056	.4067	.4078
61.0	.4089	.4101	.4112	.4123	.4134	.4145	.4157	.4168	.4179	.4191
62.0	.4202	.4214	.4225	.4237	.4248	.4260	.4271	.4283	.4295	.4306
63.0	.4318	.4330	.4342	.4353	.4365	.4377	.4389	.4401	.4413	.4425
64.0	.4437	.4449	.4461	.4473	.4485	.4498	.4510	.4522	.4535	.4547
65.0	.4559	.4572	.4584	.4597	.4609	.4622	.4634	.4647	.4660	.4672
66.0	.4685	.4698	.4711	.4724	.4737	.4750	.4763	.4776	.4789	.4802
67.0	.4815	.4828	.4841	.4855	.4868	.4881	.4895	.4908	.4921	.4935
68.0	.4948	.4962	.4976	.4989	.5003	.5017	.5031	.5045	.5058	.5072
69.0	.5086	.5100	.5114	.5129	.5143	.5157	.5171	.5186	.5200	.5214
70.0	.5229	.5243	.5258	.5272	.5287	.5302	.5317	.5331	.5346	.5361
71.0	.5376	.5391	.5406	.5421	.5436	.5452	.5467	.5482	.5498	.5513
72.0	.5528	.5544	.5560	.5575	.5591	.5607	.5622	.5638	.5654	.5670
73.0	.5686	.5702	.5719	.5735	.5751	.5768	.5784	.5800	.5817	.5834
74.0	.5850	.5867	.5884	.5901	.5918	.5935	.5952	.5969	.5986	.6003
75.0	.6021	.6038	.6055	.6073	.6091	.6108	.6126	.6144	.6162	.6180
76.0	.6198	.6216	.6234	.6253	.6271	.6289	.6308	.6326	.6345	.6364
77.0	.6383	.6402	.6421	.6440	.6459	.6478	.6498	.6517	.6536	.6556
78.0	.6576	.6596	.6615	.6635	.6655	.6676	.6696	.6716	.6737	.6757
79.0	.6778	.6799	.6819	.6840	.6861	.6882	.6904	.6925	.6946	.6968
80.0	.6990	.7011	.7033	.7055	.7077	.7100	.7122	.7144	.7167	.7190
81.0	.7212	.7235	.7258	.7282	.7305	.7328	.7352	.7375	.7399	.7423
82.0	.7447	.7471	.7496	.7520	.7545	.7570	.7595	.7620	.7645	.7670
83.0	.7696	.7721	.7747	.7773	.7799	.7825	.7852	.7878	.7905	.7932
84.0	.7959	.7986	.8013	.8041	.8069	.8097	.8125	.8153	.8182	.8210
85.0	.8239	.8268	.8297	.8327	.8356	.8386	.8416	.8447	.8477	.8508
86.0	.8539	.8570	.8601	.8633	.8665	.8697	.8729	.8761	.8794	.8827
87.0	.8861	.8894	.8928	.8962	.8996	.9031	.9066	.9101	.9136	.9172
88.0	.9208	.9245	.9281	.9318	.9355	.9393	.9431	.9469	.9508	.9547
89.0	.9586	.9626	.9666	.9706	.9747	.9788	.9830	.9872	.9914	.9957

PRINCIPLES OF EMISSION SPECTROSCOPY

I INTRODUCTION

The term "spectroscopy", in its broadest sense, refers to the study of the radiations of the electromagnetic spectrum. As no single instrument exists which will separate radiation from all parts of the spectrum, it is divided into regions which are related to the different types of instruments capable of producing or measuring waves of various lengths. A diagram of the spectral distribution of energy is shown in Figure 1.

Emission spectroscopy is concerned with that region wherein radiation can be sorted out into a spectrum by means of prisms or gratings, and includes the near infrared, the visible and the ultraviolet.

Emission spectroscopy has become an indispensable part of the modern chemist's analytical methods. With it he can analyze a wide variety of substances, both qualitatively and quantitatively, for trace elements regardless of valence states.

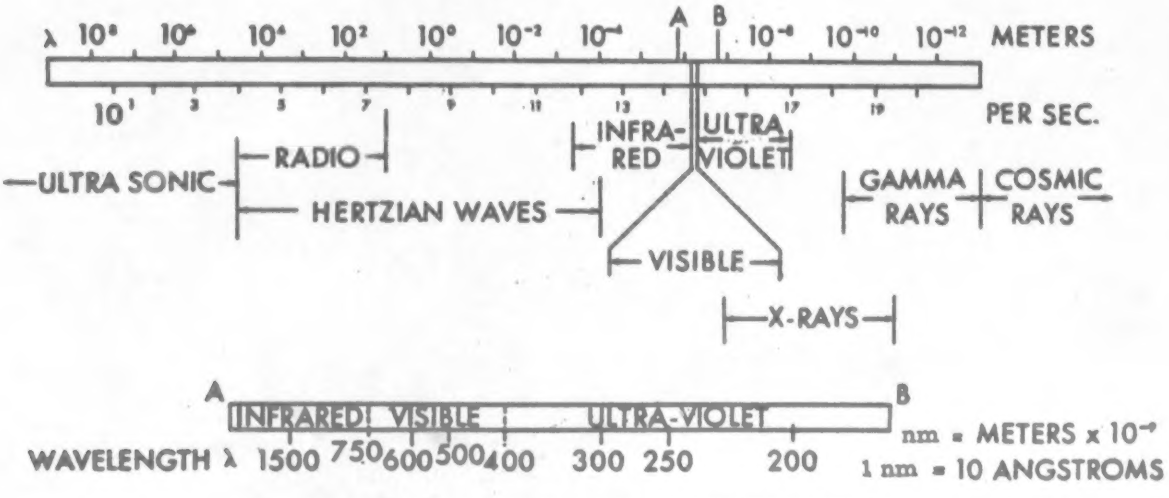
II THEORY

A Origin of Spectra - Excited atoms and atomic ions emit light of definite wavelengths. The excitation of multiple elements in a sample results in the simultaneous production of the spectra of all of these elements.

During excitation by a thermal or electrical source, the outer orbital electrons of an element absorb energy and rise to higher energy levels. These electrons then return spontaneously to their normal or ground state by a single jump or by a series of jumps. The energy emitted with each jump produces a spectral line of characteristic wave length and frequency for the particular chemical element. The combination of lines produced by the excited atoms of the element thus provides the emission spectrum of that element.

FIGURE 1

SPECTRAL DISTRIBUTION OF ENERGY


λ 10⁸ 10⁶ 10⁴ 10² 10⁰ 10⁻² 10⁻⁴
A
B
10⁻⁸ 10⁻¹⁰ 10⁻¹² METERS
10¹ 3 5 7 9 11 13 17 19
PER SEC.
INFRA-RED
ULTRA VIOLET
RADIO
ULTRA SONIC
HERTZIAN WAVES
GAMMA RAYS
COSMIC RAYS
VISIBLE
X-RAYS
A
B
INFRARED
VISIBLE
ULTRA-VIOLET
WAVELENGTH λ 1500 750 600 500 400 300 250 200
nm = METERS x 10⁻⁹
1 nm = 10 ANGSTROMS

REGION INCLUDED IN EMISSION SPECTROSCOPY

B Types of Spectra - There are three types of emission spectra.

1 The line spectra produced by highly excited atoms or atomic ions.

2 Band spectra which originate with highly excited molecules.

3 Continuous spectra which result when light is emitted by incandescent solids.

III INSTRUMENTATION

A The emission spectrograph consists of essentially four distinct functional parts: the excitation source, the slit, the optical system, and the recording system. A diagram of a Littrow type spectrograph is shown in Figure 2.

1 The production of line spectra for the detection or the determination of the elemental constituents in a sample requires an excitation source, such as an arc, a spark, or a flame.

2 The slit of a spectrograph permits only a narrow beam of light of mixed wave lengths to enter the instrument.

3 The optical system consists of a series of lenses and either a prism or grating to separate light rays of different wave lengths into the spectrum.

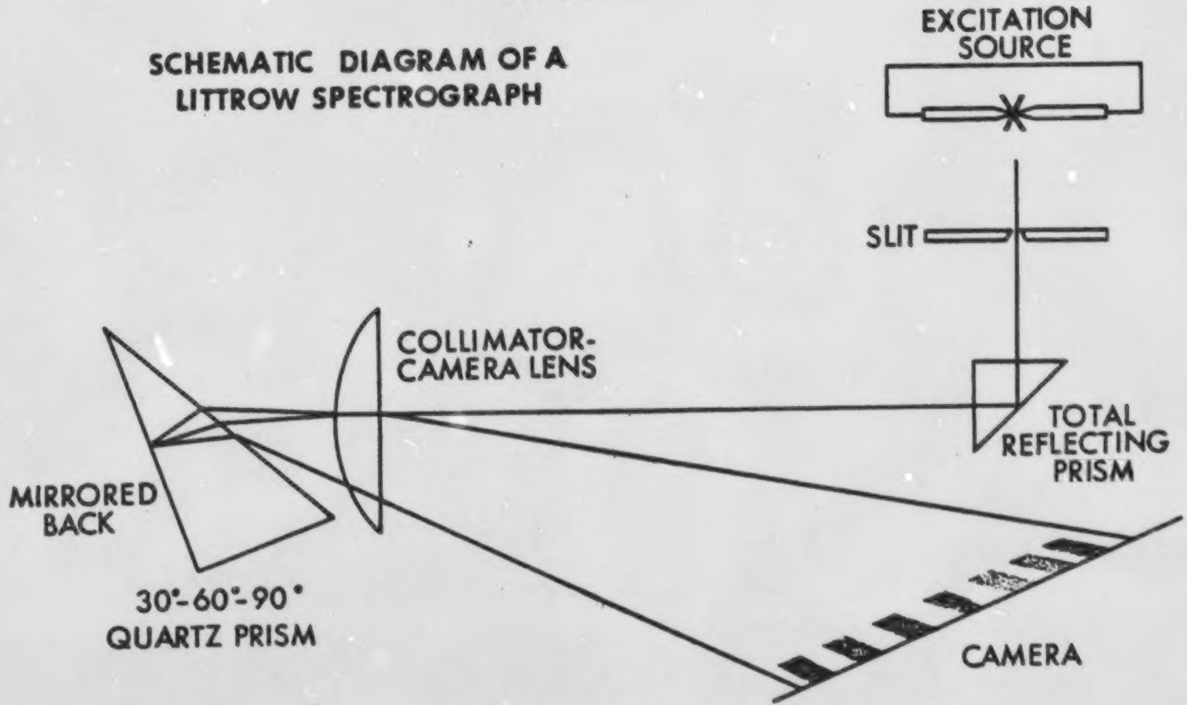
4 The recording system can be

a An eye piece such as used in a spectroscope.

b A photographic plate such as used in a spectrograph.

FIGURE 2

SCHEMATIC DIAGRAM OF A LITTROW SPECTROGRAPH



c A photoelectric cell like that used in direct reading spectrographs.

IV SELECTION OF EQUIPMENT AND COST CONSIDERATIONS

A A decision concerning the advisability of installing a laboratory for spectrochemical analysis, whether in an industrial plant, research laboratory or health department, might rest upon four main considerations.

1 Whether the analytical problems encountered have a satisfactory solution other than spectroscopy.

2 The time urgency of the work.

3 The expected volume and diversity.

4 The cost of the initial investment, together with operating expenses.

V PROBLEMS AND TECHNIQUES ASSOCIATED WITH EMISSION SPECTROSCOPY

A After the equipment has been set up properly and is ready for operating, there are many parameters to be determined before the first samples are analyzed.

1 Source - arc or spark

There are several types of excitation sources available, as shown in Table 1. Generally, the type of sample and the precision and accuracy required for the analysis will govern which excitation method will be employed.

2 Electrode

The spectrographic electrode plays an important part in the analysis. Some important features are density, shape and purity.

Table 1. EXCITATION SOURCES

TYPE	PRECISION	SENSITIVITY	RANGE	USE
D.C. ARC	Fair	Excellent	0.00001 - 1.0%	Basic sources for general qualitative analysis - soils, ores, oxides, slags, ashes, etc. Highest sensitivity of detection for trace elements.
HIGH VOLTAGE A.C. SPARK	Excellent	Fair	0.01 - 30.0%	Most stable. Use for alloying constituents in metals. Solution techniques.
LOW VOLTAGE A.C. ARC	Good	Good	0.001 - 1.0%	Quantitative determination of residual impurities for low alloying constituents in metals.
SPARK IGNITED UNI - ARC	Good	Good	0.001 - 1.0%	Combines precision of the spark and the sensitivity of the D.C. arc.
HIGH VOLTAGE A.C. ARC	Fair	Excellent	0.00001 - 1.0%	Steadier than D.C. arc - Conductors and nonrefractory materials.

a Density - The current and temperature obtainable are dependent on the density of the graphite.

b Shape - The nature of the sample being analyzed will usually determine the shape and type of electrode.

c Purity - Trace analysis requires that the electrodes be of very high purity.

3 Exposure conditions

Current, voltage, exposure time, etc., play an important part.

4 Photographic plates

The photographic emulsion most generally used for quantitative analysis is the S.A.#1. This emulsion responds to the spectral range from 2400 - 4400 A. It has high contrast, low back ground density, and low granularity.

The S.A.#2 emulsion is also desirable for trace analysis when lower contrast, higher speed, and wide latitude are needed. S.A.#2 also covers the range from 2400 - 4400 A. Other photographic emulsions covering the wavelength range from 2400 - 12,000 A are available, each having specific features. The processing of these photographic plates is extremely important and the directions of the manufacturer should be followed closely.

B Qualitative Analysis

In spectroscopy, qualitative analysis is a relatively simple process, although it can be more time consuming than a quantitative determination, especially if a complete qualitative analysis is required. The presence or absence of more than sixty of the chemical elements can be determined readily by a simple inspection of the resulting pattern of spectral lines. All elements give specific lines when sufficiently excited, which is the basis of qualitative spectrochemical analysis.

C Quantitative Analysis

Quantitative spectrochemical analysis is based on the fact that the amount of light emitted by an element present at very low concentration is directly proportional to the number of its excited atoms present, if all other factors are kept constant. The intensity of the spectral line of the analysis element (or degree of blackening of the photographed image of the line) in an unknown sample is compared to the intensity of the corresponding line in a standard sample to provide an estimate of the concentration of the element producing that line.

D Preparation of Standards

1 Since the major constituents of a sample are important factors affecting burning qualities of the arc and intensity of resulting spectral lines, it is important that the composition of the standards approximate the samples as closely as possible. This is accomplished by preparing a synthetic matrix to approximate the average composition of the samples minus the analysis element or elements.

2 Increasing concentrations of the analysis elements are added to the synthetic matrix and are sparked under the identical conditions used for the unknown samples for calibration purposes.

3 It should be emphasized that in quantitative spectrographic analysis, the best possible standards must always be prepared. A good spectrographer is only as good as his analyses, and his analyses are only as good as his standards. Spectrographic standard solutions are prepared from Reagent Grade chemicals. Oxides are prepared where possible; however, nitrates or chlorides will usually suffice.

Occasionally, it is necessary to use special spectroscopically pure grades of certain metals. Nitric and hydrochloric acids should be redistilled; double distilled water is also suggested where possible. Borosilicate glassware, cleaned with both chromic and nitric acids, is used throughout.

4 Internal standards

In quantitative analysis, the errors caused by temperature fluctuations and wandering of the are are minimized by the use of the internal standard technique. This consists of comparing the intensity of a suitable line of analysis element in a standard and in an unknown sample to a certain selected line of another element whose concentration is fixed in all samples.

Since both the unknown and the internal standards are parts of the sampe sample, variations in time exposure, plate characteristics, and developing conditions will not affect the relative density of the two lines which are equal in intensity in the light source.

Working curves are established by plotting the intensity ratio of the analysis line to the internal standared line vs. the concentration of the analysis element.

E Accuracy

The average error in quantitative emission spectroscopy, using the photographic process, is generally between ±5 - 10%. In the direct reading process, however, where photomultiplier tubes are substituted for the photographic plate, an average error of ±2% is possible. This accuracy is more than satisfactory, considering that determinations of trace elements are being made in the parts per billion range.

VI APPLICATION OF EMISSION SPECTROSCOPY TO WATER ANALYSES

A Trace elements in water originate from a variety of sources, but can be classified into three principal groups.

1 Elements contributed by soluble materials chemically weathered from rocks and soil.

2 Elements that are selectively concentrated by vegetation and find their way to surface waters following decay and run-off.

3 Industrial sources, especially those devoted to mining, alloying, and cleaning and plating of metals.

Such sources may contribute significant quantities of trace elements to surface waters in areas both populated and unpopulated. The detection and measurement of these trace elements are difficult with conventional analytical procedures because they are not adapted to large numbers of samples, and in some cases they are not sensitive enough. The use of spectrographic procedures for routine monitoring of raw waters, however, is admirably suited to the purpose since a large number of elements may be determined simultaneously with a high degree of accuracy.

B Trace elements, whether in finished or raw waters, are generally present in concentrations too low to measure directly with the spectrograph. Therefore, a means of concentration is necessary before the examination can be completed. This can be accomplished in several ways (e. g., evaporation, precipitation, ion exchange, etc.)

1 The method employed by the Environmental Protection Agency, Research and Monitoring Office, consists of evaporating a filtered volume of sample at low heat on a hot plate.

A volume of sample is chosen containing 100 mg of dissolved solids and evaporated to a volume of 5.0 ml or 20,000 mg of solids/l. (The dissolved solids consist for the most part of salts of sodium, potassium, calcium and magnesium; these are the materials for which corrections must be made later.) All samples, therefore, had the same final salt concentration, but the procedure produces varying levels of sensitivity for different sources because of the dissimilar initial volumes. Thus, the limit of detection for trace elements in the Columbia River is lower than for the Missouri River because the low dissolved solids in Columbia River waters permit a larger volume of sample to be evaporated.

a This might be explained best by examining the detection limits shown in Table 2. For convenience, we have arbitrarily chosen 400 mg/l as an average TDS content of surface water, and the detection limits as shown are based on this figure. For waters of only 200 mg TDS/l, the limit would be halved; conversely, if the TDS is 800 mg/l, the limit is doubled.

Table 2. DETECTION LIMITS*, µg/l

Zinc	20	Copper	10
Cadmium	20	Silver	2
Arsenic	100	Nickel	20
Boron	10	Cobalt	20
Iron	10	Lead	40
Molybdenum	40	Chromium	10
Manganese	10	Vanadium	40
Aluminum	40	Barium	2
Strontium	4	Beryllium	0.1

*In water having 400 mg TDS/l.

b The term "detection limit" is used to indicate the lowest concentration of the material that can be distinguished from background noise or interference. The limits, as shown in the table, are based on practical observations and are not mathematically contrived figures based on signal to noise ratios or other formulas.

c When an element has not been positively identified in a sample, the detection limit, preceded by a "less than" sign (<), is used to report the analysis. It should be emphasized that the failure to detect a particular element does not mean that the element is absent; it may be present in the original sample but in a concentration below the stated detection limit. The complete spectrographic procedure has been published previously. Table 3 lists the results from analyses of over 1500 samples. The frequency of detection, the range, and the mean concentrations are given.

2 Accuracy

Recoveries were run with a composite of samples from the Missouri River. The composite was analyzed to determine what trace elements, if any, were present and at what concentrations. To a series of aliquots of this composite were added increasing concentrations of the analysis elements. The samples were acidified, evaporated on a hot plate, transferred to cylinders, and finally sparked under identical conditions. Scaler counts were converted to concentrations by using the data obtained from the synthetic matrix and the percent recoveries were calculated. A statistical treatment of this data showed a range of 80% to 113% at the 90% confidence level.

TABLE 3. SUMMARY OF TRACE ELEMENTS IN WATERS OF THE UNITED STATES*

ELEMENT	No. of Pos. Occurrences	Frequency Of Detection, %	Observed Values, µg/l		
			Min.	Max.	Mean
Zinc	1207	76.5	2	1183	64
Cadmium	40	2.5	1	120	9.5
Arsenic	87	5.5	5	336	64
Boron	1546	98.0	1	5000	101
Phosphorus	747	47.4	2	5040	120
Iron	1192	75.6	1	4600	52
Molybdenum	516	32.7	2	1500	68
Manganese	810	51.4	0.3	3230	58
Aluminum**	456	31.2	1	2760	74
Beryllium	85	5.4	0.01	1.22	0.19
Copper	1173	74.4	1	280	15
Silver	104	6.6	0.1	38	2.6
Nickel	256	16.2	1	130	19
Cobalt	44	2.8	1	48	17
Lead	305	19.3	2	140	23
Chromium	386	24.5	1	112	9.7
Vanadium	54	3.4	2	300	40
Barium	1568	99.4	2	340	43
Strontium	1571	99.6	3	5000	217

* 1577 Samples (October 1, 1962 - September 30, 1967)
** 1464 Aluminum Samples

TABLE 4. RECOVERIES OF TRACE ELEMENTS ADDED TO RIVER WATER

Element	Percent Recovery Low	High	Average
Zn	86.	107.	94.1
Cd	100.	106.	103.5
As	80.	100.	93.5
B	82.	97.	88.9
P	70.	110.	91.8
Fe	85.	1[illegible]8.	94.1
Mo	75.	100.	83.7
Mn	94.	105.	98.1
Al	96.	125.	106.7
Be	101.	117.	109.2
Cu	96.	113.	100.8
Ag[a]			
Ni	90.	118.	103.0
Co	95.	103.	99.3
Pb	60.	93.	83.6
Cr	60.	105.	86.3
V[a]	100.	110.	104.0
Ba	70.	97.	81.7
Sr	81.	112.	95.1

[a] Silver precipitated and was not included.

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Table 4 lists the range of these per cent recoveries along with the average recovery for each element.

C Analytical Reference Service Results

A sample containing 8 trace metals at known concentrations was analyzed by 66 participating agencies using a number of analytical methods. The mean results of all 66 laboratories, in addition to those laboratories employing spectrographic procedures, are shown in Table 5. It is apparent from this summary that the spectrograph can be used to splendid advantage on water samples.

SUMMARY

The theory and instrumentation of emission spectroscopy are presented. Consideration is given to the problems and techniques associated with trace element analysis in water using the Emission Spectrograph.

For the present time, atomic absorption is the method of choice for metals analyses in the EPA Methods Manual. (8)

When broad spectrum analyses are required, the Emission Spectrograph may be used with comparable accuracy and precision.

Table 5. SUMMARY OF RESULTS OBTAINED BY SPECTROGRAPHIC PROCEDURES ON ARS SAMPLE: WATER METALS, NO. 2.

Element	Amount added mg/l	Mean of 66 Labs	AMOUNT RECOVERED mg/l		
			Lab #71126	NWQN Lab	Lab #1615
Al	1.30	2.21	1.80	2.30	3.10
Cr	0.18	0.14	0.18	0.13	0.17
Cu	0.42	0.43	0.29	0.38	0.41
Fe	0.62 **	0.44	0.46	0.40	0.75
Mn	0.25	0.28	0.25	0.23	0.38
Cd	0.24	0.26	0.25 *	0.30	0.50
Zn	0.90	0.94	0.68	0.94	0.88
Pb	0.18	0.20	0.27	0.19	--

** Iron value was adjusted to mean value as suggested on Page 47 of ARS report.

* This result was erroneously included under Lab #7112A in the ARS report.

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This outline was prepared by J.F. Kopp, Chief, Metals Analyses Unit, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Heavy Metals, Instrumentation, Metals, Spectroscopy, Water Analysis

FLAME PHOTOMETRY

I PRELIMINARY

Flame photometry is the art and science of applying thermal energy (heat) to elements in order to effect orbital shifts which produce measurable characteristic radiations. The color of the emission and the intensity of brightness of emission permit both qualitative and quantitative identification.

The application of a very hot flame (2000° C or more) produces excitation of the element, caused by the raising of an electron to a higher energy level and is followed by the loss of a small amount of energy in the form of radiant energy as the electron falls back into its original position or to a lower energy level.

II INSTRUMENTATION

The six essential parts of a flame photometer are: pressure regulators and flow meters for the fuel gases, atomizer, burner, optical system, photosensitive detector and an instrument for indicating or recording output of the detector. These components are schematically shown in Figure 1.

A Atomizer and Burner

Numerous variations in atomizer and burner designs have been used. Figure 2 depicts the integral aspirator-burner used in Beckman instruments. The sample is introduced through the innermost concentric tube, a vertical

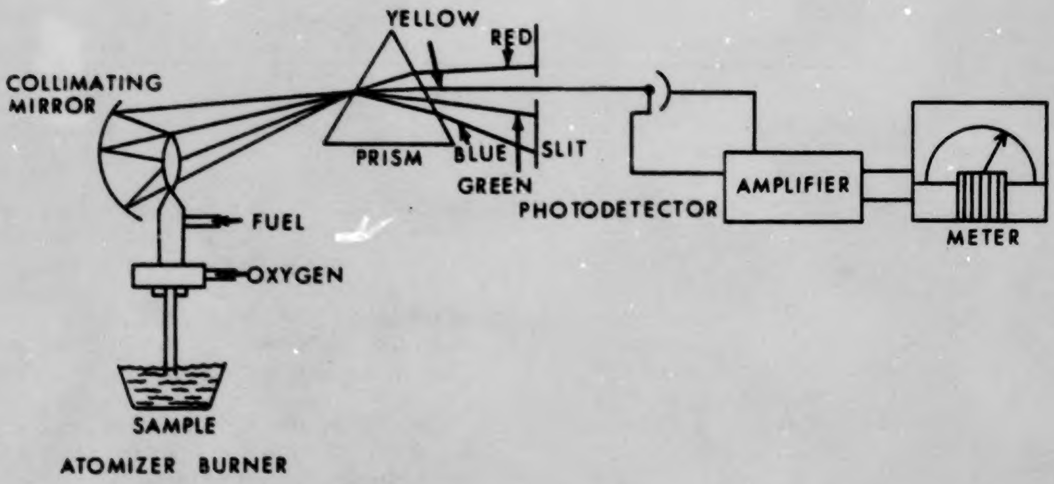

FIGURE 1. SIMPLIFIED DIAGRAM OF A FLAME PHOTOMETER

palladium capillary. A concentric channel provides oxygen, and its tip is constricted to form an orifice. Oxygen is passed from this orifice causing the sample solution to be drawn up to the tip of the inner capillary. There, the liquid is sheared off and dispersed into droplets. All droplets are introduced directly into flame, with a sample consumption of 1 - 2 ml per minute.

The main requirement of the burner is production of a steady flame when supplied with fuel and oxygen or air at constant pressures. In the Beckman aspirator-burner, a concentric channel provides oxygen to operate the atomizer and the flame. The additional concentric channel provides fuel for the flame.

B Optical System, Photosensitive Detector and Amplifier

The optical system must collect the light from the steadiest part of the flame, render it monochromatic with a prism, grating or filters, and then focus it onto the photosensitive surface of the detector. Use of filter photometers is least desirable due to their limited resolution. Flame spectrophotometers improve application as they will separate emissions in a mixture of metals, such as manganese lines at 403.3 nm and the potassium lines at 404.6 nm Placement of a concave mirror behind the flame so that the flame is at the center of the curvature increases intensity of flame emission by a factor of 2.

Any photosensitive device may be used in a flame photometer. The detector must have a response in the portion of the spectrum to be used and have good sensitivity. The photomultiplier tube is the preferred detector for flame spectrophotometers.

The amplifier increases the signal from the phototube and improves resolution between close spectral lines. It also permits identification of elements present in samples when the concentration is very small.

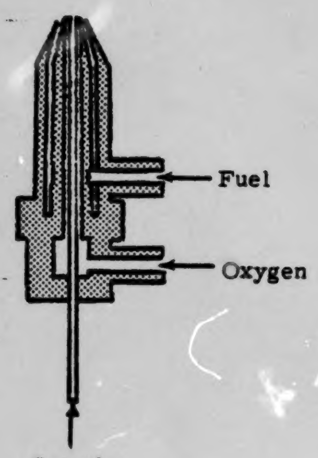

Figure 2. DETAILED DIAGRAM OF BURNER-ATOMIZER

III APPLICATIONS OF FLAME PHOTOMETRY TO WATER ANALYSES

Measurement of sodium and potassium in the past has been confined to complex, tedious and time-consuming gravimetric procedures. The flame technique enables the analyst to perform these determinations in a matter of seconds. If these metals alone were the only elements capable of measurement by flame photometry the use of the instrument could still be justified in a great many laboratories.

Other cations which may be detected and measured in waters and waste materials are calcium, magnesium, lithium, copper, and others. Table 1 includes those elements which may be measured with commercially available equipment, including ultra-violet and photomultiplier accessories.

Table 1 does not include wavebands which occur in the infrared spectrum. Sodium, for example, has an emission band at 819 nm which is not detectable with the common instruments.

Many other metals, including the rare earths, can be measured using the flame technique but they are not included in the table because

Table 1

	Wavelength	Approximate Sensitivity mg/1		Wavelength	Approximate Sensitivity mg/1
Aluminum	484.2	2	**Lead**	405,	2
	467.2	3		368$^{?}$,	2
	396.2	4		364$^{?}$	3
Barium	553.6	0.3	**Lithium**	670.8	0.002
	493	0.4			
			Magnesium	371,	0.1
Beryllium	471	25		383$^{?}$	0.1
	510	100		285.2	0.2
Boron	548	1	**Manganese**	403	0.01
	521	2		279*	1
	495	3		561	2
Cadmium	326.1$^{?}$	2	**Mercury**	235.7*	10
	228.8*	40			
			Potassium	766.5	0.001
Calcium	422.7	0.003		404.6,	0.2
	632	0.004		344.7$^{?}$	3
	554	0.01			
			Silver	338.3$^{?}$,	0.05
Chromium	425.4	0.1		328.1$^{?}$	0.1
	360$^{?}$	0.1			
	520	0.1	**Sodium**	589.3,	0.002
				330.3$^{?}$	1
Copper	324$^{?}$	0.01			
	327$^{?}$	0.01	**Strontium**	460.7	0.02
				681	0.01
Iron	372	0.2		407.8	0.5
	386	0.2			
	373	0.3	**Zinc**	213.9*	500
				500	200

* = Ultra-violet spectrum

? = Doubtful detection in visible spectrum

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the necessity for their measurement in water is a rare occurrence.

IV INTERFERENCES

A Spectroscopic Interferences

Energy at other wave lengths or from other elements than those intended to be measured may reach the detector. This problem is related to the resolution of the instrument and slit widths used.

Many of the instrumental difficulties are related to reproducibility of the flame. The quality and composition of the fuel affect the constancy and temperature of the flame which in turn influences the energy of emission. Likewise, slight variations in fuel pressures and ratios affect the reproducibility of the flame with reference to shape, temperature, background, rate of sample consumption, etc. In some cases, the temperature of the flame is the limiting factor in determining the presence of a metal. (The alkaline earth metals emit radiations at "low" temperatures, whereas other metals require very "hot" flames.)

Table 2 indicates temperatures obtainable with different fuel-oxidant mixtures.

Table 2.
Approximate Temperatures of
Fuel-Oxidant Mixtures for
Flame Photometer Use

Fuel-Oxidant	Approximate Temp. °C
Hydrogen - air	2100
Hydrogen - oxygen	2700 - 2800
Acetylene - oxygen	3100
Acetylene - air *	2000 - 2200
Propane - oxygen	2700 - 2800
Illuminating gas - oxygen	2800
Cyanogen - oxygen **	4900

* Undesirable because of carbon deposits.
** Used in research problems.

Emission reading of spectral lines always includes any contribution from the flame background emission on which the line is superimposed. When the photometer includes a monochromator, it is possible to read the background radiation in the presence of the test element. First, the line + background intensity is measured in the normal manner at the peak or crest of the band system. Next, the wave length dial is rotated slowly until emission readings decrease to a minimum at a wave length located off to one side or the other of the emission line or band. It is usually preferable to read the background at a lower wave length than the peak. Background reading is subtracted from the line + background reading.

Products of combustion may affect the characteristics of the flame or may affect the optical system by fogging or coating of lenses and mirrors.

B Factors Related to the Composition of the Sample

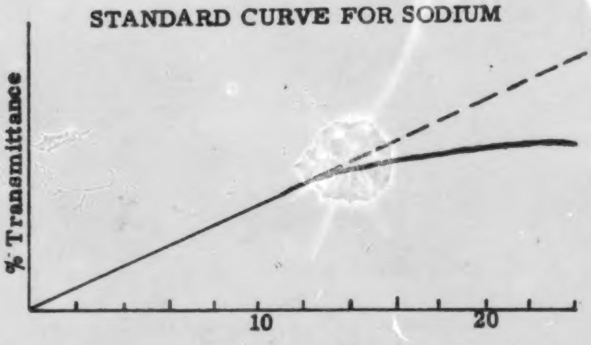
An element may be self-absorbing -- a phenomenon in which the energy of excitation is not proportional to the concentration of the element. As previously discussed, exictation is followed by loss of energy in the form of radiation as the electron falls back to its original position or to a lower energy level. During passage of radiant energy through the outer fringes of the flame, this energy is subject to absorption through collision with atoms of its own kind present in the ground energy level. Absorption of radiant energy weakens the strength of the spectrum line. Using the emission line at 589 nm for sodium, Figure 3 indicates that the line ceases to be linear at 13 mg/l. As the sodium concentration increases, the self-absorption effects become more pronounced. Sample dilution to permit reading on linear portion of the curve is often practiced.

Two or more elements present in the sample may produce radiant energy at the same, or near the same wavelength. For instance, calcium at 423 nm and chromium at 425 nm could interfere with each other by additive effect. The correction may be to dilute out the unwanted metal or measure one of the emissions at a different wavelength.

The emission energy of one element may be enhanced or depressed by energies from other elements. This phenomenon (radiation interference) occurs when one element causes another to modify its actual emission intensity in either a negative or positive manner. Correction is obtained by dilution or by controlled interference addition.

Other types of difficulties encountered are too numerous to list here. In general, they may be overcome by improved instruments (high resolution, narrower slit openings, optics, flame adjustment) or possibly by special techniques.

Some inexpensive instruments, designed for limited use, may employ illuminating gas with air or propane with air as a matter of economy or convenience.



STANDARD CURVE FOR SODIUM

Figure 3

V TECHNIQUES

The following techniques are intended to serve as examples of current procedures in use for routine samples and for special samples where corrective procedures are indicated.

A Emission Intensity vs. Concentration

This is the classical procedure in flame photometry. Solutions (standards) containing known concentrations of test elements are compared with an unknown sample. This technique is applicable only when no interference is present.

B Radiation Buffers

For measurements of alkaline earth metals (sodium, potassium, calcium, magnesium) radiation buffers are prepared as solutions saturated with regard to each metal, respectively. A potassium buffer, for example, is prepared by saturating distilled water with sodium, calcium, and magnesium chloride. A calcium buffer in turn is saturated with sodium, potassium and magnesium chloride.

C Preparation of Radiation Buffers

For a sodium measurement, the buffer solution is added equally to samples and standards so that the interferences are alike for all readings, thereby cancelling each other (see Table 3).

D Instrument Improvement

Potassium emits energy bands at 766, 405, and 345 nm. The bands are at opposite ends of the spectrum and the 405 and 345 bands are not usable in the visible spectrum. The 766 line also loses sensitivity because of its proximity to the infrared region. Use of a red sensitive phototube or photomultiplier, however, permits measurement with an ordinary instrument at concentrations as low as 0.1 mg/l, or less. This approach is applicable to other elements also.

E Standard Addition

Equal volumes of the sample are added to a series of standard solutions containing different known quantities of test element, all diluted to the same volume (see Table 4). Emission intensities of the resulting solutions are then determined at the wavelength of maximum emission and at a suitable point on the flame background. After subtracting the background emission, the resulting net emissions are plotted linearly against the concentration of the increments of the standard solutions that were mixed with the unknown. The percent transmission of the mixture containing unknown sample and zero standard (distilled water) is doubled and the concentration corresponding to this point on the graph will be the concentration of the undiluted unknown sample. This can be explained algebraically in conjunction with Figure 4.

F Internal-Standard Method

The method consists of adding to each sample and standard a fixed quantity of internal standard element. The element must be one not already present in the sample. Lithium is usually the internal standard used. This method is most convenient when using instruments having dual detectors. The emission intensities of standards and samples are read simultaneously or successively depending upon instrumentation.

G Separation of Interferences

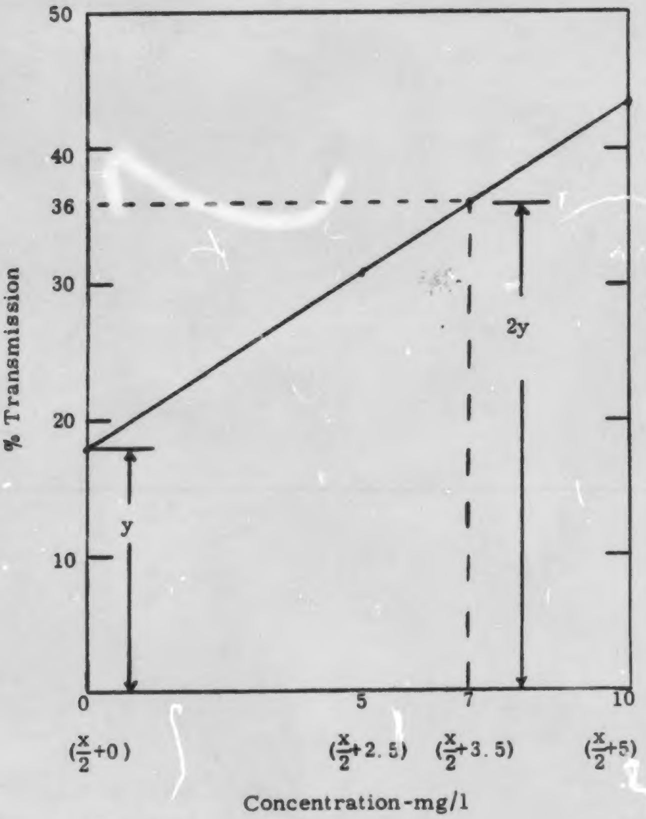
In cases where certain elements interfere, they may be physically removed, or the interference may be "blocked out" by reading the emission at different wavelengths. To measure lithium, for example, calcium, barium, and strontium are precipitated as carbonates of the metals. The lithium is retained in the filtrate and measured at a wavelength of 671 nm.

	NaCl	KCl	$CaCl_2$	$MgCl_2$
Sodium Buffer	-	+	+	+
Potassium Buffer	+	-	+	+
Calcium Buffer	+	+	-	+
Magnesium Buffer	+	+	+	-

Table 3

Conc. of standards	0.0 mg/l	5.0 mg/l	10.0 mg/l
Volume of standard added to sample	10.0 ml	10.0 ml	10.0 ml
Volume of sample used	10.0 ml	10.0 ml	10.0 ml
Concentration of element in each portion of mixture	$\frac{x}{2}$ + 0 mg/l	$\frac{x}{2}$ + 2.5 mg/l	$\frac{x}{2}$ + 5 mg/l

Table 4



Let x = concentration of element in unknown sample.

Then Y = % transmission of an equal mixture of unknown sample and zero standard, or

$Y = \frac{x}{2} + \frac{0}{2}$ which simplifies to $2Y = x$

$\therefore\ 2Y = \frac{x}{2} + 3.5$ (from the example in Figure 4)

by substitution, $x = \frac{x}{2} + 3.5$

$\frac{x}{2} = 3.5$

$x = 7$ mg/l

Figure 4

16-7

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This outline was prepared by R. C. Kroner, Chief, Physical and Chemical Methods, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Water Tests, Flame Photometry, Spectrophotometry, Spectroscopy

FLAME PHOTOMETRY LABORATORY (SODIUM)

I REAGENTS

A Deionized Distilled Water

To be used for the preparation of all reagents, calibration standards, and as dilution water.

B Stock Sodium Solution

Dissolve 2.542 g of NaCl, previously dried at 140°C, in deionized distilled water and dilute to 1000 ml.

1.00 ml = 1.00 mg Na^+

C Intermediate Sodium Solution

Dilute 10.00 ml of the stock sodium solution to 100.0 ml with deionized distilled water. Use this solution for preparing the calibration curve in the sodium range of 1-10 mg/l.

1.00 ml = 100 μg Na^+

D Standard Sodium Solution

Dilute 10.00 ml of the intermediate sodium solution to 100 ml with deionized distilled water. Use this solution for preparing the calibration curve in the sodium range of 0.1-1.0 mg/l.

1.00 ml = 10.0 μg Na^+

II INTERFERENCE CONTROL

Refer to the cited reference

III STANDARDS

Standards may be prepared in any of these applicable ranges: 0 - 1.0, 0 - 10, or 0 - 100 mg/l.

IV INSTRUMENT OPERATING CONDITIONS

Theoretical wavelength 589 nm

Fuel pressure 7.5 lbs/in^2

Oxygen pressure 10 lbs/in^2

For all other conditions needed, consult the manufacturer's instrument manual.

V PROCEDURE

1 Number the six plastic cups provided 0, 2, 4, 6, 8, and 10.

2 Fill them 3/4 full with the appropriate sodium standards; e.g., 0 mg/l standard into cup 0, etc.

3 Fill a 7th plastic cup 3/4 full with the unknown.

4 Fill an 8th plastic cup with distilled water.

5 The power toggle switch (on left side of instrument) is already turned on.

6 Set the sensitivity knob to the standby position.

7 Set the wavelength knob to the theoretical valve of 589 nm (the scale is at the top of the instrument).

8 The fltr - shtr - open knob is in the shtr (closed) position.

9 Open the main valve on the oxygen cylinder; all other oxygen gauges are already set.

10 Open the main valve on the hydrogen cylinder; all other hydrogen gauges are already set.

11 Raise the door on the right side of the burner housing (behind instrument).

12 Cautiously bring a lighted match to the tip of the burner in the housing.

13 Close the door on the burner housing.

14 Caution: Do not place any part of your body over the coils on the top of the burner housing. Hot gases are escaping.

15 Raise the silver lever between the instrument and the burner housing to the vertical position.

16 Place the plastic cup containing distilled water in the cup holder which is now exposed at the right side of the burner housing.

17 Push the silver lever clockwise so that the cup holder swings into the burner housing and the water is aspirated. A distinct difference in sound will be noticed when water or a sample is being aspirated. If at any time during the determination this sound again changes, it will indicate that all of the liquid has been aspirated from the cup. Simply move the silver lever and refill the cup with the appropriate liquid.

18 Do not allow air to be aspirated for more than about 15 seconds. If there is any delay, aspirate distilled water until the problem causing the delay has been corrected.

19 Turn the sensitivity knob to position 1.

20 Turn the dark current knob until the needle reads 0 on the percent transmittance scale.

21 Turn the sensitivity knob to position 4.

22 Repeat step 20.

23 Swing the cup of distilled water out of the burner housing and replace it with cup 10. Swing this cup back into the burner housing.

24 Turn the fltr - shtr - open knob to the open position (this opens the shutter).

25 Turn the wavelength knob slowly to the left; the needle will move to the left.

26 At some point the needle will suddenly swing toward the right. It will probably be necessary to make adjustments with the slit knob in order to keep the needle on-scale while finding the point at which the needle swings back to the right. Record this wavelength. It is the peak wavelength. Do not change this setting until indicated in the instructions.

27 If the point at which the needle swings back to the right is overshot, turn the wavelength knob about 1/4 turn to the right and repeat steps 25 and 26.

28 Make the needle read 100% transmittance by turning the slit knob. Record the slit mm reading. Do not change this setting. 100 is the peak transmittance reading for this solution.

29 Turn the fltr - shtr - open knob to the shtr position (this closes the shutter).

30 Replace cup 10 with cup 8.

31 Open the shutter.

32 Record the percent transmittance reading and close the shutter.

33 Repeat steps 30, 31, and 32 using cups 6, 4, 2, 0, and the unknown, in turn.

34 Aspirate distilled water and using the dark current knob make the needle read 0 percent transmittance.

35 Aspirate cup 10.

36 Open the shutter.

37 Slowly turn the wavelength knob to the left. The needle will move to the right and at about 1/4 - 1 percent transmittance will move no further to the right. Record the wavelength reading. This is the background wavelength. Do not change this setting. The percent transmittance is the background reading for this solution.

38 If the point at which the needle moves no further to the right is overshot, turn the wavelength about 1/4 turn to the right and repeat step 37.

39 Close the shutter.

40 Replace cup 10 with cup 8.

41 Open the shutter.

42 Record the background transmittance reading for this solution.

43 Close the shutter.

44 Repeat steps 40, 41, 42, and 43 using cups 6, 4, 2, 0, and the unknown, in turn.

45 Aspirate distilled water for about 15 sec.

46 Turn the sensitivity knob to the standby position.

47 Close the main valve on the hydrogen cylinder.

48 Close the main valve on the oxygen cylinder.

49 Empty the eight plastic cups and discard them.

50 Leave the power toggle switch (on left side of instrument) on.

51 For each of the 6 solutions subtract the background percent transmittance reading from the peak percent transmittance reading.

52 Using the graph paper provided in the manual, plot the 6 differences vs. the appropriate concentrations. Draw the line to best fit connecting the 6 points. This is the calibration graph.

53 Find the difference percent transmittance for the unknown on the percent transmittance axis.

54 Draw a straight line to the right until it intersects the calibration line.

55 From the point of intersection draw a line straight down to the concentration axis.

56 This is the concentration of the unknown.

REFERENCE

Standard Methods for the Examination of Water and Wastewater, 13th Edition, page 317, Method 153A. 1971.

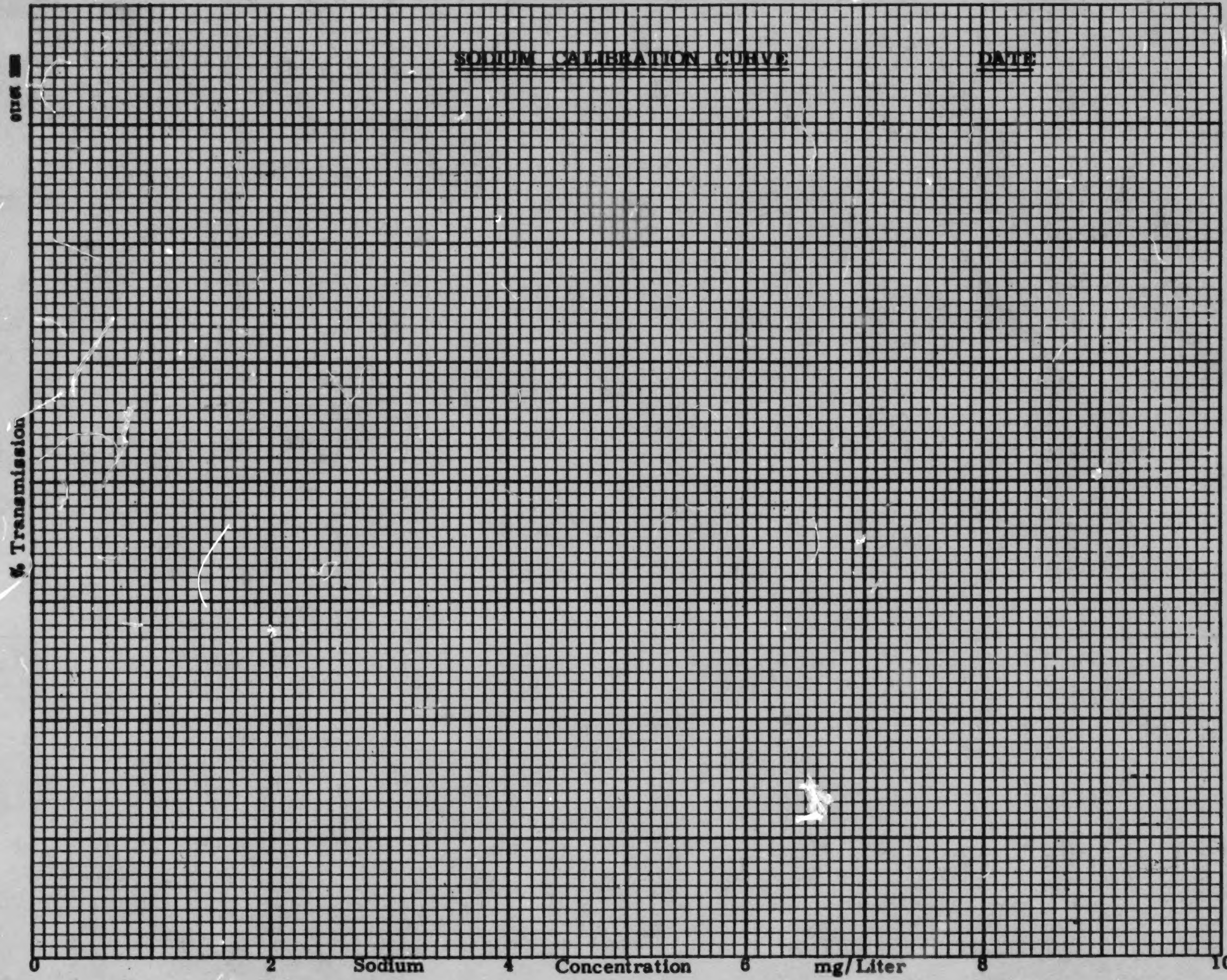
Percent Transmission Readings

	Background	Peak	Difference
0.0 mg/l	______	______	______
2.0 mg/l	______	______	______
4.0 mg/l	______	______	______
6.0 mg/l	______	______	______
8.0 mg/l	______	______	______
10.0 mg/l	______	______	______
Sample	______	______	______

Peak Wavelength ______________ **nm**

Background Wavelength __________ **nm**

Slit _____ **mm**


SODIUM CALIBRATION CURVE
DATE
% Transmission
0
2
Sodium
4
Concentration
6
mg/Liter
8
10

17-5

This outline was prepared by C. R. Feldmann, National Training Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Laboratory Tests, Water Analysis, Sodium, Alkali Metals, Metals

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FLAME PHOTOMETRY LABORATORY (STRONTIUM)

I GENERAL

This procedure is listed as being "tentative" in the cited reference. Also, strontium is not listed in Table I of the Federal Register, Volume 38, Number 199, Tuesday, October 16, 1973; i.e., as of October 16, 1973, strontium is not included in the National Pollutant Discharge Elimination System.

II REAGENTS

A Fifty percent by volume hydrochloric acid

B NH_4OH, 3N

C Stock Strontium Solution

Weigh 1.685 g of anhydrous $SrCO_3$ and place it in a 500 ml Erlenmeyer flask. Place a small funnel in the neck of the flask and add 50% HCl slowly until all of the $SrCO_3$ has dissolved. Add 200 ml of distilled water and boil for a few minutes to expel CO_2. Cool and add a few drops of methyl red indicator. Adjust to the intermediate orange color by adding 50% by volume HCl or 3N NH_4OH. Transfer quantitatively to a 1 liter volumetric flask and dilute to the mark with distilled water.

1.00 ml = 1.00 mg Sr^{+2}

D Standard Strontium Solution

Dilute 25.00 ml of stock strontium solution to 1000 ml with distilled water. Use this solution for preparing Sr standards in the 1 - 25 mg/l range.

1.00 ml = 25.0 μg Sr^{+2}

III INTERFERENCE CONTROL

The radiation effect of possible interfering substances is equalized throughout the standards by use of the standard addition technique.

IV INSTRUMENT OPERATING CONDITIONS

Theoretical wavelength 460.7 nm

Fuel pressure 7.5 lbs/in^2

Oxygen pressure 10 lbs/in^2

For all other conditions needed, consult the manufacturer's instrument manual.

V PROCEDURE

1 Number the five flasks provided 0, 5, 10, 15, and 20.

2 Into each of the five, pipette 10.0 ml of the unknown.

3 Using a clean pipette, add 10.0 ml of the 0 mg/l standard into flask 0.

4 Again using a clean pipette, add 10.0 ml of the 5 mg/l standard into flask 5.

5 Proceed in a similar manner using the 10, 15, and 20 mg/l standards and flasks 10, 15, and 20.

6 Stopper and shake all five flasks.

7 Mark five plastic cups 0, 5, 10, 15, and 20.

8 Fill the plastic cups about 3/4 full with the appropriate solutions from the flasks.

9 Fill a 6th plastic cup with distilled water.

10 The power toggle switch (on left side of instrument) is already turned on.

11 Set the sensitivity knob to the standby position.

12 Set the phototube voltage knob (on right side of instrument) to position C.

13 Set the wavelength knob to the theoretical value of 461 nm (the scale is at the top of the instrument).

14 Set the fltr - shtr - open knob in the shtr position (closed position).

15 Open the main valve on the oxygen cylinder; all other oxygen gauges are already set.

16 Open the main valve on the hydrogen cylinder; all other hydrogen gauges are already set.

17 Raise the door on the right side of the burner housing (behind instrument).

18 Cautiously bring a lighted match to the tip of the burner in the housing.

19 Close the door on the right side of the burner housing.

20 Caution: Do not place any part of your body over the coils on the top of the burner housing. Hot gases are escaping.

21 Raise the silver lever between the instrument and the burner housing to the vertical position.

22 Place the plastic cup containing distilled water in the cup holder which is now exposed at the right side of the burner housing.

23 Push the silver lever clockwise so that the cup holder swings into the burner housing and the water is aspirated. A distinct difference in sound will be noticed when water or a sample is being aspirated. If at any time during the determination this sound again changes, it will indicate that all of the liquid has been aspirated from the cup. Simply move the silver lever and refill the cup with the appropriate liquid.

24 Do not allow air to be aspirated for more than about 15 sec. If there is any delay, aspirate distilled water until the problem causing the delay has been corrected.

25 Turn the sensitivity knob to position 1.

26 Turn the dark current knob until the needle reads 0 on the percent transmittance scale.

27 Turn the sensitivity knob to position 4.

28 Repeat step 26.

29 Swing the cup of distilled water out of the burner housing and replace it with cup 20. Swing this cup back into the burner housing.

30 Turn the fltr - shtr - open knob to the open position (this opens the shutter).

31 Turn the wavelength knob slowly to the left; the needle will move to the left.

32 At some point the needle will suddenly swing toward the right. It will probably be necessary to make adjustments with the slit knob in order to keep the needle on-scale while finding the point at which the needle swings back to the right. Record this wavelength. It is the peak wavelength. Do not change this setting until indicated in the instructions.

33 If the point at which the needle swings back to the right is overshot, turn the wavelength knob about $\frac{1}{4}$ turn to the right and repeat steps 31 and 32.

34 Make the needle read 100% transmittance by turning the slit knob. Record the slit mm reading. Do not change this setting. 100 is the peak percent transmittance reading for this solution.

35 Turn the fltr - shtr - open knob to the shtr position (this closes the shutter).

36 Replace the cup 20 with 15.

37 Open the shutter.

38 Record the percent transmittance reading.

39 Close the shutter.

40 Repeat steps 36, 37, 38, and 39, using cups 10, 5, and 0 in turn.

41 Aspirate distilled water and using the dark current knob make the needle read 0 percent transmittance.

42 Aspirate cup 20.

43 Open the shutter.

44 Slowly turn the wavelength knob to the left. The needle will move to the right and at about 5 - 30 percent transmittance will move no farther to the right. Record the wavelength reading. This is the background wavelength. Do not change this setting. The percent transmittance is the background reading for this solution.

45 If the point at which the needle moves no farther to the right is overshot, turn the wavelength about $\frac{1}{4}$ turn to the right and repeat step 44.

46 Close the shutter.

47 Replace cup 20 with cup 15.

48 Open the shutter.

49 Record the background transmittance reading for the solution.

50 Close the shutter.

51 Repeat steps 47, 48, 49, and 50 using cup 10, 5, and 0 in turn.

52 Aspirate distilled water for about 15 sec.

53 Turn the sensitivity knob to the standby position.

54 Close the main valve on the hydrogen cylinder.

55 Close the main valve on the oxygen cylinder.

56 Empty all six plastic cups and discard them. Empty the flasks.

57 Leave the power toggle switch (on left side of instrument) on.

58 For each of the 5 solutions, subtract the background percent transmittance reading from the peak percent transmittance reading.

59 Using the graph paper provided in the manual, plot the 5 differences vs. the appropriate concentrations. Draw a straight line connecting the five points. This is the calibration graph.

60 Double the difference value obtained for the solution in cup 0.

61 Find the value on the percent transmission axis.

62 Draw a straight line to the right until it intersects the calibration line.

63 From the point of intersection, draw a line straight down to the horizontal axis.

64 This is the concentration of the unknown.

REFERENCE

Standard Methods for the Examination of Water and Wastewater, 13th Edition, page 328, Method 155A. 1971.

	Percent Transmission Readings		
	Background	Peak	Difference
10.0 ml unknown + 10.0 ml 0.0 mg/l std.			
10.0 ml unknown + 10.0 ml 5.0 mg/l std.			
10.0 ml unknown + 10.0 ml 10.0 mg/l std.			
10.0 ml unknown + 10.0 ml 15.0 mg/l std.			
10.0 ml unknown + 10.0 ml 20.0 mg/l std.			

Peak Wavelength ______________ nm

Background Wavelength ________ nm

Slit __________________________ mm

ANALYSIS OF UNKNOWN STRONTIUM SOLUTION
DATE
% Transmission
0
5
Standard
10
Addition
15
mg/Liter
20

This outline was prepared by C. R. Feldmann, National Training Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Chemical Analysis, Laboratory Tests, Water Analysis, Strontium, Alkaline Earth Metals, Metals

AUTOMATION OF CHEMICAL ANALYSIS

I INTRODUCTION

Environmental pollution control and monitoring have become, in very recent years, the focal point of national and international attention. The analytical chemist has an important role in matters regarding pollution, for he determines not only the amount of pollution in air or water but also the efficiency of prevention and treatment methods. The increasing number of samples and of measurements to be made requires him to employ every means possible to keep abreast with the demands.

The need for instrumental and automated analytical techniques is well recognized. Automated sensors with continuous readout for the measurement of key constituents associated with pollution would be ideal.

II DEGREES OF AUTOMATION

A Maximum automation - continuous sampling direct from source, with telemetering of results to central computer.

B Medium automation - grab samples in laboratory with multiple simultaneous instrumental analysis, recording, calculating and digital print-out of data.

C Minimum automation - maximum simplicity and efficiency of manual analytical methods.

Possible considerations are:

1 Automatic pipettes, burettes and reagent dispensers

2 Choice of glassware - precision and accuracy of graduated test tubes and cylinders vs. volumetric flasks should be compared to the accuracy of the analytical method.

3 Electrical "on and off" timers on hot plates (C.O.D.) and automated instruments (Technicon).

4 Use of semi-micro methods:

- a TKN - digest and distill 50 ml sample in place of 500 ml.
- b B.O.D. - incubate 125 ml samples in place of 300 ml.

5 Combining reagents:

- a Nitrite - reagent contains buffer, diazotization and coupling reagent.
- b Phosphorus - reagent contains acid, molybdate and reductant.

6 Laboratory management - elimination of needless repetitious work

- a Lab's sample log number vs. engineer's coding system
- b Distributing samples to instrumental stations in permanent labeled containers
- c Permanent data sheets vs. individual slips of paper for record keeping.
- d Washing glassware - cleaning acid vs. soap; labor costs
- e Documented laboratory procedures, techniques, and practices to avoid variations.

III SENSORS AND INSTRUMENTATION

In order to measure the amount of any constituent in a liquid or gaseous medium on an automated system, a relationship between the parameter and an electrical signal must be established. When such a relationship is established and is not affected by uncontrollable variables, electronic technology already available can be applied to the sensor's signal for digital print-out of concentration or for telemetering and conversion of the signal to meaningful data through computerization.

Various types of sensors and instrumental methods for water quality parameters are shown in Table 1. Parameters whose measurement is based on an electrochemical principle are very desirable because of the possibility of developing an electrode or probe. In such a case, no intermediate chemical reaction is needed for the ion in the sample to produce a signal.

A Probes for many specific ions are available; however, most are affected by interferences and variables. The activity of the specific ion, sensed by the electrodes, is related to its concentration by the activity coefficient, which depends on the total ionic strength of the solution(4). By increasing the ionic strength in all the samples, the effect of individual variation between samples can be eliminated.

With some specific ion probes, a pH adjustor and a complexing agent must also be added to the samples to eliminate interference from hydrogen, hydroxyl and metal ions.

Specific ion probes are valuable in some applications but must be evaluated for such use. Most of the automated continuous monitoring systems for water quality that are now available use sensors which require no intermediate chemical reaction and only measure parameters such as temperature, pH, conductivity, solar radiation, turbidity, chlorides and dissolved oxygen.

B Automatic Titrators with preset end point are applicable for many measurements using various types of electrodes. Up to 10 samples can be titrated without attendance.

C Atomic Absorption - This method is applicable for determining the concentration of many metals. The instrument is usually used only in a laboratory. It is adaptable to:

1 an automatic samples
2 automatic reagent additions
3 automatic extractions

D Photometric Methods - With automated addition of reagents, the end product can be measured with flow through cells in:

1 Visible range

2 Ultra violet range

E Organic Carbon Analyzer - Manual injection of samples vs. automated sampling system is dependent on amount of suspended solids in samples.

IV PRESERVATIVES AND QUALITY CONTROL

A Preservatives added to samples must be compatible with the automated method to be used. For example, sulfuric acid and mercury may interfere with colorimetric methods.

With automated methods, the preservative should be added to samples, standards and control blanks.

B Routine standardization of automated instrument - After every 10 or 20 samples:

1 Check baseline with a control blank.

2 Check sensitivity with a standard

The laboratory should also establish a program for routine interlaboratory and intralaboratory quality control.

V AUTOMATED WET-CHEMICAL SYSTEMS

Many chemical parameters for water quality are defined by the conditions in the chemical analytical methodology of Standard Methods, e.g. C.O.D., chlorine, phenolics and TKN digestion. All new automated methods should be compared to the manual Standard Method for accuracy.

A Robot Chemist - individual sample and reaction vessel, syringe type measurement of reagents, micro-switches and values, complex mechanics. Many are not very well accepted by chemists.

B Mecolab - individual sample and reaction containers; maximum 15 samples without manual change

C Technicon AutoAnalyzer - a continuous flow through system propelled by depression of various inside diameters of tygon tubing using constant speed rollers. The modular system is capable of almost all manual chemical techniques, such as sampling (up to 200 samples), filtration, dilution, addition of reagents, mixing, heating, dialysis, steam distillation, extraction with an immiscible solvent and digestion. The concentration of the end products is measured with a photometer or fluorometer and recorded. The versatility of the AutoAnalyzer enables the automation of almost any manual procedure.

The success of each automated procedure is primarily dependent on the manifold design. Effort should be made to achieve the maximum rate of each chemical reaction by determining the optimum conditions of time, temperature, pH and reagent concentration. When this is obtained, there is generally an increase in sensitivity, thus requiring a smaller aliquot of sample with less chance of interfering ions, a shorter flow stream through the system which will produce a faster response and wash out (shorter sampling time) and smoother, steadier plateaus.

The Technicon AutoAnalyzer is now internationally used for automating wet-chemical procedures in the laboratory. The Analytical Quality Control Laboratory of EPA includes Technicon procedures in the manual(9) of methods for Office of Water Programs laboratories.

VI CONTINUOUS MONITORING SYSTEM

A Several criteria or objectives have been established for using the AutoAnalyzer for continuous monitoring of a stream or a treatment plant influent or effluent:

1 Capability of continuous operation for one week with a minimum amount of manual manipulation (less than 4 man hours per week).

2 At least a ± 5% accuracy at the 50% (0.30 absorbance) absorption concentration.

3 Reagents and base line should remain stable for a week.

4 Reagent consumption should be less than 4 liters per week.

5 There should be a minimum distance of reaction-flow stream for a fast response and equilibrium of the system.

6 It is desirable that colorimetric chemistry obeys Beer's Law for rapid calculations.

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(Continued on Page 35-5)

TABLE 1

INSTRUMENTAL METHODS FOR ANALYSIS OF WATER QUALITY PARAMETERS

PARAMETER	SENSOR	MODE OF MEASUREMENT	USES*	EVALUATION
TEMPERATURE	probe	thermacouple	P, F, L, C	excellent
pH	electrode	potentiometric	P, F, L, C	good
CONDUCTIVITY	electrode	conductivity	P, F, L, C	good
SOLAR RADIATION	photocell	photometric	P, F, L, C	excellent
DISSOLVED OXYGEN	electrode	polarographic	P, F, L, C	good
CHLORIDE	electrode	conductivity	P, F, L, C	fair
	wet-chemical	potentiometric photometric	F, L, C L	— —
TURBIDITY	photocell (scatter)	photometric	P, F, L, C	good
COLOR	photocell (absorbance)	photometric	F, L, C	good
SUSPENDED SOLIDS	photocell	photometric	—	—
ALKALINITY	wet-chemical	potentiometric	L	—
	wet-chemical	photometric	L	—
ACIDITY	wet-chemical	potentiometric	L	—
	wet-chemical	photometric	L	—
CALCIUM	electrode	potentiometric	P, L	—
	flame	atomic absorption	L	—
	wet-chemical	photometric	L	—
MAGNESIUM	flame	atomic absorption	L	—
	wet-chemical	photometric	L	—
AMMONIA	electrode	potentiometric	P, L, C(?)	—
	wet-chemical	photometric	P, L, C(?)	good
NITRITE	wet-chemical	photometric	P, L, C(?)	good
NITRATE	probe	potentiometric	—	—
	wet-chemical	photometric	P, L, C	good
PHOSPHORUS	wet-chemical	photometric	P, L, C	good
SULFATE	wet-chemical	photometric	L	—
SULFIDE	wet-chemical	photometric	L	—

TABLE 1 CONTINUED

INSTRUMENTAL METHODS FOR ANALYSIS OF WATER QUALITY PARAMETERS

PARAMETER	SENSOR	MODE OF MEASUREMENT	USES*	EVALUATION
TOTAL ORGANIC CARBON	wet-chemical	infrared	L	—
	wet-chemical	flame ionization	L	—
CYANIDE	electrode	potentiometric	P	—
	wet-chemical	photometric	P, L, C(?)	—
PHENOLICS	wet-chemical	photometric	—	—
SPECIFIC ORGANICS	gas-chromatography	electron-capture	L	—
	liquid-chromotography	photometric	L	—
BACTERIOLOGICAL	wet-enzyamatic	photometric	—	—
	wet-fluorescence	fluorometric	—	—

*F-Field, monitoring streams
P-Treatment Plants, monitoring influent or effluent
L-Laboratory, with individual or automated sampling system
C-Continuous operation, for monitoring streams or treatment plants for operational control.

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Descriptors: Analysis, Automation, Chemical Analysis, Instrumentation, Water Analysis

TURBIDITY

I INTRODUCTION

Turbidity as a water quality index refers to the degree of cloudiness present. Conversely, it is an index of clarity.

A Definition(1)

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through samples of water.

B Relationship to Suspended Solids

This optical property, turbidity, is caused by suspended matter. The size, shape and reflection/absorption properties of that matter (not its weight) determine the degree of optical effects. It is very possible to have water with high turbidity but very low mg/l suspended solids. Thus one cannot use turbidity results to estimate the weight concentration and specific gravity of the suspended matter.

C Causes

1 clay, sand

2 silt, erosion products

3 microscopic and macroscopic organisms

4 finely divided organic products

5 others

D Effects on Water Quality(2)

Turbidity is an indicator of possible suspended matter effects such as impeding effective chlorine disinfection and clogging fish gills. However, the following list is limited to those effects associated with the optical (clarity) nature of turbidity.

1 Reducing clarity in water

- a drinking water quality
- b food processing
- c industrial processes
- d fish (seeing natural food)
- e swimming/water sports

2 Obscuring objects in water

- a submerged hazards
- b water sports

3 Light penetration

- a affects depth of compensation point for photosynthetic activity (primary food production).

4 Thermal Effects

High turbidity causes near surface waters to become heated because of the heat absorbancy of the particulate matter.

- a Results in lower rate of oxygen transfer from air to water.
- b Stabilizes water column and prevents vertical mixing.
    - 1 decreases downward dispersion of dissolved oxygen
    - 2 decreases downward dispersion of nutrients

E Criteria for Standards(2)

1 Finished Drinking Water - Maximum of one unit where the water enters the distribution system. The proposed(3) standard is one unit monthly average and five units average of two consecutive days. Under certain conditions a five unit monthly average may apply at state option.

2 For Freshwater Aquatic Life and Wildlife - The combined effect of color and turbidity should not change the

compensation point more than 10% from its seasonally established norm, nor should such a change place more than 10% of the biomass of photosynthetic organisms below the compensation point.

3 Turbidity Criteria Used by Industries:

a Textiles - 0.3 to 5 units

b Paper and allied products - Ranges from 10 to 100 units, depending on type of paper.

c Canned, dried and frozen fruits and vegetables - Same as for finished drinking water (1 turbidity unit).

F Processes to Remove Turbidity (Solids)

1 Coagulation

a pre-chlorination enhances coagulation

2 Sedimentation

3 Filtration

4 Aeration

5 Others

II VISUAL METHODS TO ESTIMATE TURBIDITY

A Early Efforts

In the early 1900's, Whipple and Jackson measured turbidity and developed a calibration scale for turbidity instruments.

B Jackson Candle Turbidimeter

Later Jackson developed apparatus which utilized the same "extinction" principle as the instrument devised earlier with Whipple.

1 Instrument

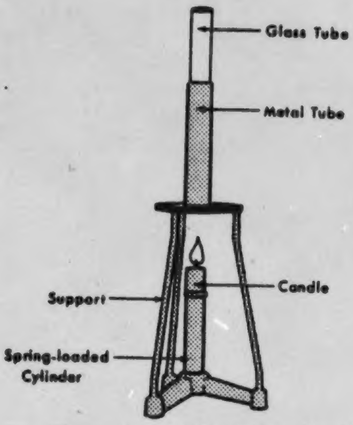

Figure 1 JACKSON CANDLE TURBIDIMETER

The sample was poured into a flat-bottomed, graduated glass tube held over a special candle. A turbidity reading was taken when the operator, observing from the top of the tube, saw the image of the candle flame disappear into a uniform glow. The reading related the final depth of sample in the tube with tube calibrations obtained from a standard suspension solution.

2 Standard Suspension

The standard was a suspension of silica prepared from Fuller's or diatomaceous earth. This was diluted to prepare a series of standard suspensions to graduate the turbidimeter. Graduations on all Jackson turbidimeters are made in conformity to this original data. Other suspensions are standardized by using the pre-calibrated turbidimeter and diluting accordingly.

3 Unit Used

Jackson Turbidity Unit (JTU) - parts per million suspended silica turbidity.

4 Standardization of Apparatus

The current edition of Standard Methods[1] contains specifications for the three essential components, i.e., the calibrated glass tube, the candle and a support.

5 Current Standard Suspension Solutions[1]

1 Natural turbid water from the same source as that tested gives best results. Determine turbidity with the instrument, then dilute to values desired.

2 The supernatant of a settled solution of kaolin is also used as a standard.

6 Limitations of Method

a Apparatus - difficult to exactly reproduce flame as to intensity and actual light path length. In general, it is a rather crude instrument with several variables that affect accuracy.

b Very fine suspended particles do not tend to scatter light of the longer wavelengths produced by the candle.

c Very dark and black particles can absorb enough light in comparison to the scattering of light to cause an incorrect reading of image extinction.

d Turbidities below 25 JTU cannot be directly measured. For lower turbidities (as in treated waters), indirect secondary methods are required to estimate turbidities.

C Hellige Turbidimeter[4]

This instrument utilizes the same extinction principle as the Jackson Candle Turbidimeter.

1 Equipment

An opal glass bulb supplies the light which is reflected (usually through a filter) upward through the sample which is contained in a glass tube. The entire system is enclosed in a black metal box. The operator views the sample by looking downward through an ocular tube screwed into the top of the box and adjusts the brightness of a central field of light by turning a calibrated dial on the outside of the apparatus. The point of uniform light intensity occurs when a black spot in the center of the field just disappears.

2 Range of Applicability

The equipment offers a choice of bulbs, filters and volumes of sample tubes. The variety affords a means to directly measure turbidity ranging from 0 through 150. The ranges can be extended by dilution.

3 Results

The final reading from the dial is translated into ppm silica turbidity units by using a graph corresponding to the bulb, filter and volume of sample used.

4 Standard Suspension Solution

Standardizing suspensions are not used by the operator. The graphs are supplied by the company for each instrument.

D Secchi Disk[5]

This is a very simple device used in the field to estimate the depth of visibility (clarity) in water.

1 Equipment

The disk is a weighted circular plate, 20 cm in diameter, with opposing black and white quarters painted on the surface. The plate is attached to a calibrated line by means of a ring on its center to assure that it hangs horizontally.

2 Readings

The disk is lowered into water until it disappears, lowered farther, then raised until it reappears. The corresponding visibility depth (s) are determined from the calibrated line. Some read both depths and average them. Some read only the reappearance depth.

3 Standardizing the Procedure

There are many variables (position of sun and of observer, roughness of body of water, etc.) that affect readings. However, the same observer using a standard set of operating conditions can provide useful data to compare the visibility of different bodies of water.

4 Application of Results

Limnologists have found it convenient to establish a Secchi disk "factor" for estimating the photic depth where light intensity is about 1 per cent of full sunlight intensity. The true photic depth is determined by use of a submarine photometer and at the same time the observer takes a series of Secchi disk readings to obtain an average. Dividing the true photic depth by this average gives a factor which can be used to multiply other disk readings for an approximation of photic depth.

E Status of Visual Methods for Compliance Monitoring

The Federal Register(6) "List of Approved Methods" does not include any of these visual methods for National Pollutant Discharge Elimination System (NPDES) requirements. The visual methods are not recognized in the Federal Register(3) issue on Interim Drinking Water Regulations, either.

III NEPHELOMETRIC MEASUREMENTS FOR COMPLIANCE MONITORING

The subjectivity and apparatus deficiencies involved in visual methods of measuring turbidity make each unsuitable as a standard method.

Since turbidity is an expression of the optical property of scattering or absorbing light, it was natural that optical instruments with photometers would be developed for this measurement.

The type of equipment specified for compliance monitoring(3, 6) utilizes nephelometry.

A Basic Principle(7)

The intensity of light scattered by the sample is compared (under defined conditions) with the intensity of light scattered by a standard reference solution (formazin). The greater the intensity of scattered light, the greater the turbidity. Readings are made and reported in NTUs (Nephelometric Turbidity Units).

B Schematic

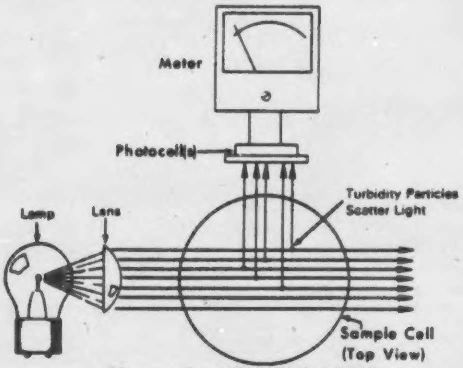

Figure 2 NEPHELOMETER (90° Scatter)

Light passes through a polarizing lens and on to the sample in a cell. Suspended particles (turbidity) in the sample scatter the light.

Photocell (s) detect light scattered by the particles at a 90° angle to the path of the incident light. This light energy is converted to an electric signal for the meter to measure.

1 Direction of Entry of Incident Light to Cell

a The lamp might be positioned as shown in the schematic so the beam enters a sample horizontally.

b Another instrument design has the light beam entering the sample (in a flat-bottom cell) in a vertical direction with the photocell positioned accordingly at a 90° angle to the path of incident light.

2 Number of Photocells

The schematic shows the photocell (s) at one 90 degree angle to the path of the incident light. An instrument might utilize more than one photocell position, with each final position being at a 90 degree angle to the sample liquid.

3 Meter Systems

a The meter might measure the signal from the scattered light intensity only.

b The meter might measure the signal from a ratio of the scattered light versus light transmitted directly through the sample to a photocell.

4 Meter Scales and Calbration

a The meter may already be calibrated in NTUs. In this case, at least one standard is run in each instrument range to be used in order to check the accuracy of the calibration scales.

b If a pre-calibrated scale is not supplied, a calibration curve is prepared for each range of the instrument by using appropriate dilutions of the standard turbidity suspension.

C EPA Specifications for Instrument Design(7)

Even when the same suspension is used for calibration of different nephelometers, differences in physical design of the turbidimeters will cause differences in measured values for the turbidity of the same sample. To minimize such differences, the following design variables have been specified by the U. S. Environmental Protection Agency.

1 Defined Specifications

a Light Source

Tungsten lamp operated at not less than 85% of rated voltage and at not more than rated voltage.

b Distance Traveled by Light

The total of the distance traversed by the incident light plus scattered light within the sample tube should not exceed 10 cm.

c Angle of Light Acceptance of the Detector

Detector centered at 90° to the incident light path and not to exceed ± 30° from 90°.

(Ninety degree scatter is specified because the amount of scatter varies with size of particles at different scatter angles).

d Applicable Range

The maximum turbidity to be measured is 40 units. Several ranges will be necessary to obtain adequate coverage. Use dilution for samples if their turbidity exceeds 40 units.

2 Other EPA Design Specifications

a Stray light

Minimal stray light should reach the photocell (s) in the absence of turbidity.

Some causes of stray light reaching the photocell (s) are:

1 Scratches or imperfections in glass cell windows.

2 Dirt, film or condensation on the glass.

3 Light leakages in the instrument system.

A schematic of these causes is shown in Figure 3.

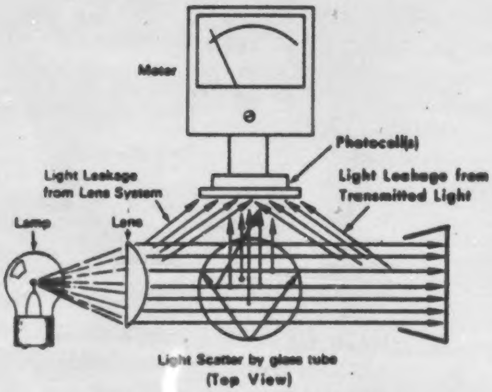
Meter
Photocell(s)
Light Leakage from Lens System
Light Leakage from Transmitted Light
Lamp
Lens
Light Scatter by glass tube
(Top View)

Figure 3 NEPHELOMETER SOURCES OF STRAY LIGHT

Stray light error can be as much as 0.5 NTU. Remedies are close inspection of sample cells for imperfections and dirt, and good design which can minimize the effect of stray light by controlling the angle at which it reaches the sample.

b Drift

The turbidimeter should be free from significant drift after a short warm-up period. This is imperative if the analyst is relying on a manufacturer's solid scattering standard for setting overall instrument sensitivity for all ranges.

c Sensitivity

In waters having turbidities less than one unit, the instrument should detect turbidity differences of 0.02 unit or less. Several ranges will be necessary to obtain sufficient sensitivity for low turbidities.

3 Examples of instruments meeting the specifications listed in 1 and 2 above include:

a Hach Turbidimeter Model 2100 and 2100 A

b Hydroflow Instruments DRT 100, 200, and 1000

4 Other turbidimeters(12) meeting the listed specifications are also acceptable.

D Sources of Error

1 Marred Sample Cells

a Discard scratched or etched cells.

b Do not touch cells where light strikes them in instrument.

c Keep cells scrupulously clean, inside and out.(8)

1 Use detergent solution.

2 Organic solvents may also be used.

3 Use deionized water rinses.

4 Rinse and dry with alcohol or acetone.

2 Standardizing Suspensions(7)

a Use turbidity - free water for preparations. Filter distilled water through a 0.45 μ m pore size membrane filter if such filtered water shows a lower turbidity than the distilled water.

b Prepare a new stock suspension of Formazin each month.

c Prepare a new standard suspension and dilutions of Formazin each week.

3 Sample Interferences

a Positive

1 Finely divided air bubbles

b Negative

1 Floating debris

2 Coarse sediments (settle)

3 Colored dissolved substances (absorb light)

E Reporting Results(7)

NTU	Record to Nearest:
0.0-1.0	0.05
1-10	0.1
10-40	1
40-100	5
100-400	10
400-1000	50
>1000	100

F Precision and Accuracy(7)

1 In a single laboratory (MDQARL), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard deviations were ± 0.60, ± 0.94, ± 1.2 and ± 4.7 units, respectively.

2 Accuracy data is not available at this time.

IV STANDARD SUSPENSIONS AND RELATED UNITS(9)

One of the critical problems in measuring turbidity has been to find a material which can be made into a reproducible suspension with uniform sized particles. Various materials have been used.

A Natural Materials

1 Diatomaceous earth

2 Fuller's earth

3 Kaolin

4 Naturally turbid waters

Such suspensions are not suitable as reproducible standards because there is no way to control the size of the suspended particles.

B Other Materials

1 Ground glass

2 Microorganisms

3 Barium sulfate

4 Latex spheres

Suspensions of these also proved inadequate.

C Formazin

1 A polymer formed by reacting hydrazine sulfate and hexamethylenetetramine sulfate.

2 It is more reproducible than previously-used standards. Accuracy of ± one per cent for replicate solutions has been reported.

3 In 1958, the Association of Analytical Chemists initiated a standardized system of turbidity measurements for the brewing industry by:

a defining a standard formula for making stock Formazin solutions and

b designating a unit of measurement based on Formazin, i.e., the Formazin Turbidity Unit (FTU).

4 During the 1960's Formazin was increasingly used for water quality turbidity testing. It is the currently recognized standard for compliance turbidity measurements.

D Units

1 At first results were translated into Jackson Turbidity Units (JTU). However, the JTU was derived from a visual measurement using concentrations (mg/liter) of silica suspensions prepared by Jackson. They have no direct relationship to the intensity of light scattered at 90 degrees in a nephelometer.

2 For a few years, results of nephelometric measurements using specified Formazin standards were reported directly as Turbidity Units (TU's).

3 Currently, the unit used is named according to the instrument used for measuring turbidity. Specified Formazin standards are used to calibrate the instrument and results are reported as Nephelometric Turbidity Units (NTUs).

V TURBIDITY MEASUREMENTS FOR PROCESS CONTROL

The schematic and design characteristics discussed above for nephelometric instruments is the required method for measuring turbidity for compliance purposes. Turbidity data is also widely used to check water for process design purposes and to monitor water for process control purposes. The nature of the liquids to be monitored, and the degree of sensitivity required for signalling the remedy to be applied have led to the development of monitoring instrumentation that differs in design or in principle from the instrument previously described.

A Users of Control Data

1 Potable Water Treatment Plants

2 Municipal Wastewater Treatment Plants

3 Industrial Processers

B Applications of Control Data (10, 11)

1 Coagulation Processes

a To check the effectiveness of different coagulants.

b To check the effectiveness of different dosages.

c To regulate chemical dosages by automating chemical feed controls.

2 Settling Processes

a To determine intermittent need for settling processes.

b To control the sludge blanket height in activated sludge treatment processes.

c To activate removal and re-cycling of very high density sludge from settling tanks.

d To monitor effectiveness of settling processes.

3 Filtration Processes

a To determine intermittent need for filtration.

b To facilitate high rate filtration processes.

c To prevent excessive loadings for filtration systems.

d To check the efficiency of filtration systems.

e To regulate filter backwash operations.

4 Rust in Water Distribution Systems

a To locate sources of contamination.

b To monitor intermittent occurrences.

5 Steam Boiler Operations

a To detect corrosion products in boiler water.

b To detect evidences of corrosion in condensates.

c To determine the effectiveness of corrosion treatment measures.

C Varieties of Instrumentation

1 Surface Scatter Nephelometers

In forward - scattering instruments, the angle of the incident light is adjusted to illuminate the surface of a smooth flowing liquid at an angle of about 15 degrees from horizontal, rather than beamed through a glass cell of the liquid as described for a nephelometer earlier in this outline. A photocell is located immediately above the illuminated area so that vertically scattered light from turbidity in the sample reaches it.

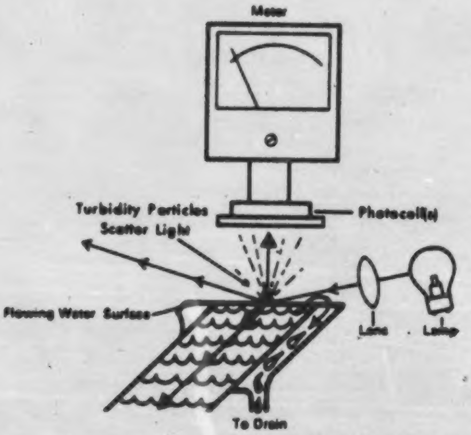

Figure 4 NEPHELOMETER (Surface Scatter)

Variations of the methodology include sidescatter and backscatter designs.

a Advantages

1 No glass sample cells are used. Attendant problems of cleanliness and condensation are eliminated. The surface of the liquid provides a near-perfect optical surface which is difficult to achieve in glass cells.

2 Stray light effects on the photocell are minimized because the simpler design eliminates some of the sources of stray light.

3 Since flowing sample is used, interferences from air bubbles and/or floating materials are quickly eliminated.

4 This design is sensitive to the presence of larger suspended particles.

b Disadvantage

As turbidity becomes high, penetration of incident light decreases to cause a falling off of response.

2 Absorption Spectrophotometry

The incident light is beamed through a smooth, flat stream of sample and the transmitted light (in contrast to nephelometric scattered light) is measured by a spectrophotometer. A schematic is shown in Figure 5.

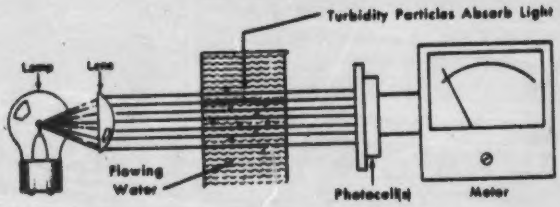

Figure 5 ABSORPTION SPECTROPHOTOMETRY

a Advantages

1 No glass sample cells are used.

2 The simpler design eliminates sources of stray light.

3 Applicable to measure high turbidities, e.g., in sludges.

b Disadvantage

1 Low sensitivity for many applications.

2 Color constituents interfere.

VI SUMMARY

Turbidity measurements represent the optical property of light scattering by suspended solids. NTUs are an index of the effects of the size, etc., of suspended particles but cannot be used to indicate mg/l quantities of those particles. The parameter is required for finished potable water and is extremely useful in reference to aesthetic quality (clarity), photic conditions and thermal effects in bodies of water. It is also widely applied for process control of water and wastewater treatment and of industrial processes.

There have been difficulties in developing a satisfactory standard method for this measurement. Early methods depended on a subjective judgement of an extinction point where transmitted light balanced scattered light in rather crude apparatus. Although the apparatus was refined and standardized to a large extent, the subjectivity of these visual methods was still an unsatisfactory element of such methodology.

Eventually, optical instrumentation was developed to eliminate subjectivity from the measurement. Nephelometry (scattering) was chosen for the standard method and U. S. EPA has specified several instrument design criteria to further promote standardization of the measurement.

Finding a suitable (reproducible) standard suspension has also been a problem. Currently, Formazin is specified as the standard because, to date, it is more reproducible than other suspensions proved to be.

Establishing a meaningful unit progressed along with development of instrumentation and agreement on a standard suspension. The current unit (NTU) is derived from the method of measurement, nephelometry, and use of a standard Formazin suspension.

Even with the efforts to standardize instrument design, to find a suitable standard suspension, and to agree on a meaningful unit, there are still problems about this measurement. Instruments meeting the design criteria and standardized with Formazin suspensions can give turbidity readings differing significantly for the same sample.

Another problem area is associated with sample dilutions. Work has indicated a progressive error on sample turbidities in excess of 40 units, so such samples are to be diluted. However, obtaining a dilution exactly representative of the original suspension is difficult to achieve. Thus dilutions often significantly fail to give linearly decreased results when re-measured.

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12 Bausch and Lomb and Turner of California are additional examples (9/77) of Models meeting the listed specifications.

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Descriptors: Chemical Analysis, Instrumentation, Secchi disks, Turbidity, Wastewater, Water Analysis

CALIBRATION AND USE OF A TURBIDIMETER (NEPHELOMETER)

I REAGENTS(1)

A Turbidity - free water - Pass distilled water through a 0.45 µm pore size membrane filter if such filtered water shows a lower turbidity than the original distilled water.

B Stock Turbidity Suspension - 400 units

1 Directions are for preparing 100.0 ml. Larger volumes may be required.

2 Solution, 1: Dissolve 1.00g hydrazine sulfate, $(NH_2)_2 \cdot H_2SO_4$, in turbidity free water and dilute to 100ml in a volumetric flask.

3 Solution 2: Dissolve 10.00g hexamethylenetetramine in turbidity-free water and dilute to 100ml in a volumetric flask.

4 Suspension: Mix 5.0ml Solution 1 with 5.0ml Solution 2 in a 100 ml volumetric flask. Allow to stand for 24 hours at 25 ± 3°C. Dilute to the mark and mix.

5 Stability: Prepare a new stock suspension each month.

C Standard Turbidity Suspension - 40 units

1 Dilute 10.00ml stock turbidity suspension to 100ml with turbidity - free water in a 100 ml volumetric flask. The turbidity is defined as 40 units.

2 Stability: Prepare a new standard suspension each week.

D Dilute Standard Turbidity Suspension - 4 units

1 Dilute 1.0 ml stock turbidity suspension to 100 ml with turbidity-free water in a 100 ml volumetric flask. The turbidity should be 4 units.

2 Stability: Prepare a new standard suspension each week.

E Secondary Standards

1 Solutions standardized with Formazin can be purchased from the manufacturer of the instrument.

2 Date such solutions. Store under the conditions specified. Discard and replace when flocculation in the solution is observed or when it fails a periodic check with a Formazin Standard. (2)(3)

II PREPARATIONS FOR MEASUREMENTS

A Suspensions

1 Check date of preparation and prepare fresh solutions if required.

B Sample Cell

1 Cells should be cleaned immediately after use as described in V.B. below.

2 Inspect cells for cleanliness. If necessary, clean them using V.B. below.

3 Check cells for scratches and etching. Discard those with imperfections.

C Instrument

1 Scale - If a scale is inserted, check that it is in the correct position. If the scale is blank, construct a calibration scale for each range on the instrument. (See III B).

2 Zero - Adjust meter needle to zero point on scale as directed by manufactuer.

3 Lens - Check for cleanness. If required, follow manufacturer's instructions for removing and cleaning the lens. Accurate re-positioning of the lens is critical for accurate measurements.

4 Warm-Up Period - Follow manufacturer's instructions. Continuous running is often suggested because of the photomultiplier tubes.

5 Focus - Use template or method described by manufacturer to check and, if necessary, set the focus.

D Determine Range of Sample Turbidity

1 Use steps 5 through 16 in III A below EXCEPT Step 12 which should be: Obtain a turbidity reading from the scale.

2 Note which Range (instrument scale) best "brackets" the turbidity of each sample.

3 If the turbidity of a sample exceeds 40 units, use the higher scales provided to determine the dilution required so the final reading will be below 40 units. For final measurements, use the Range (Scale) appropriate for the diluted sample.

III INSTRUMENT CALIBRATION AND MEASUREMENTS(1)

A Pre-Calibrated Scale

1 Each day, prepare at least one standard for the required instrument range (s) (as determined in II. D. above) by diluting one of the Formazin suspensions described above in I, Reagents. The table below gives "EXAMPLE DILUTIONS".

2 Set the instrument RANGE knob at the first range to be tested. (The instrument should be ON, warmed up, zeroed, etc., as in II C above).

3 Make any instrument adjustment specified by the manufacturer to use this RANGE.

4 Rinse the SAMPLE CELL 3 times with the appropriate suspension (or sample).

5 Shake the suspension to thoroughly disperse the solids. (For secondary standards, check the manufacturer's instructions for this step).

EXAMPLE DILUTIONS

NO.	EXAMPLE INSTRUMENT RANGES	VOLUME	TURBIDITY STANDARD	FINAL DILUTION	FINAL TURBIDITY
1	0 - 0.1	2.5 ml	4 unit	100.0 ml	0.1
2	0 - 0.2	5.0 ml	4 unit	100.0 ml	0.2
3	0 - 0.3	7.5 ml	4 unit	100.0 ml	0.3
4	0 - 1	2.5 ml	40 unit	100.0 ml	1
5	0 - 3	7.5 ml	40 unit	100.0 ml	3
6	0 - 10	25.0 ml	40 unit	100.0 ml	10
7	0 - 30	7.5 ml	400 unit	100.0 ml	30
8	0 - 100	25 ml	400 unit	100.0 ml	100
9	0 - 300	75 ml	400 unit	100.0 ml	300
10	0 - 400	100 ml	400 unit	100.0 ml	400
11	0 - 1000	100 ml	400 unit	100.0 ml	400

6 Wait until air bubbles disappear in the suspension.

7 Pour the suspension into the SAMPLE CELL up to the level specified by the manufacturer. CAUTION: Always hold the cell above the area from which light scattering is measured.

8 If applicable, screw cap on cell.

9 Wipe the outside of the cell with a lint-free tissue.

10 Examine the suspension in the cell to check for air bubbles. If air bubbles are present, eliminate them:

  a by inserting the cell in the sample holder and waiting a few minutes so bubbles rise above photomultiplier tube. CAUTION: More bubbles can form if a temperature rise occurs.

  b by holding the cell at the top and:

    1 flicking side with your finger or

    2 dipping the end of the cell into an ultrasonic cleaning bath or

    3 centrifuging the filled cell in cups with rubber cushions and surrounded with water.

    4 NOTE: After any of these remedies, again wipe the outside of the cell. When air bubbles are gone, insert the cell in the sample holder.

11 Place the LIGHT SHIELD according to the manufacturer's instruction.

12 Use the STANDARDIZING control to obtain a meter reading corresponding to the turbidity of the standard suspension.

13 Remove the LIGHT SHIELD.

14 Remove the SAMPLE CELL.

15 Discard the standard suspension.

16 Rinse SAMPLE CELL 3 times with turbidity - free water.

17 Use Steps 4 through 11 for each sample (or diluted sample) to be tested in this range. For samples, step 12 should be : Record the turbidity reading for the sample. Then do Steps 13 through 16 as above.

NOTE: The final reading for samples should not exceed 40 NTU. If this reading is exceeded for a sample, dilute it and repeat the calibration/measurement procedure above using the appropriate range and standard. (Selection of the range as described in II. D. above should make this unnecessary at this stage of the procedure).

B Non-Calibrated Scale

Prepare a series of standards and make a calibration scale for each range of the instrument.

  a The instrument should be ON, warmed up, zeroed, etc., as in II C above.

  b Prepare enough standards to give several points on each scale so estimated readings can be reasonably accurate.

  c Use the table of EXAMPLE DILUTIONS in III A above to prepare the highest standard for each instrument range. The rest of each calibrating series can also be prepared by dilutions based on the information in the table.

2 Self-prepared scales should also be calibrated each day using the procedure given in III A above for pre-calibrated scales.

3 Self-prepared scales are used for samples in the manner described in III A above for pre-calibrated scales.

IV CALCULATION/RESULTS(1)

A Diluted Samples

Multiply final sample readings by the appropriate dilution factor.

B Reporting Results

Report results as follows:

NTU	Report to Nearest:
0.0 - 1.0	0.05
1 - 10	0.1
10 - 40	1
40 - 100	5
100 - 400	10
400 - 1000	50
> 1000	100

C Precision and Accuracy

1 In a single laboratory (MDQARL), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard deviations were ± 0.60, ± 0.94, ± 1.2 and ± 4.7 units, respectively.

2 Accuracy data is not available at this time.

V STORAGE

A Standard Suspensions

1 Store in glass containers at room temperature.

2 Excess light or heat may affect stability.

3 Observe stability times noted for each in I. above.

B Sample Cells

1 Discard cells with scratches or etching.

2 Clean cells immediately after use with this order of treatments:(4)

a detergent

b organic solvents, if required

c deionized water

d alcohol or acetone rinses to dry

e lint-free tissue, if required

3 Store in a manner to protect the cells from scratches.

C Instrument

1 A line operated instrument should be permanently located so moving it often is not necessary.

2 Turbidimeters should be protected from dust, especially the lens system.

3 Store any removable parts as directed by manufacturer.

4 Close any access doors.

5 Because of the photomultiplier tubes, the manufacturer may suggest continuous running of the instrument to insure maximum accuracy for measurements. Frequency of use can determine the actual routine for warm-up time.

6 Follow any other storage directions in the manufacturer's manual.

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Descriptors: Analytical Techniques, Laboratory Tests, Turbidity

TRACE ORGANIC CONTAMINANTS IN WATER

I INTRODUCTION

The subject of trace organic contaminants in water continues to receive increasing amounts of attention. Concurrently, the sources of these refractory materials are becoming more varied and complex. The problems associated with these substances are likewise increasing and satisfactory water treatment is becoming more and more difficult.

II SOURCES

A Man Made

1 Domestic wastes - In various stages of sewage treatment, discharged into rivers and streams.

2 Many complex industrial wastes, which have been accidentally or deliberately spilled or dumped.

3 Carrier solvents, such as those used in pesticide formulations.

4 Chemicals, such as fertilizers, which are applied directly to the land and water.

5 Petrochemicals.

B Natural

1 Extracellular products of algae by

  a Diffusion of metabolic intermediates;

  b By-products of metabolism;

  c Hydrolysis of capsular materials.

2 Actinomycetes - microorganisms present in rivers/streams by their growth and decomposition cycles.

3 Bacterial decomposition of organic materials.

4 Run-off of land vegetation and soil.

5 Sediments, through decomposition and subsequent exchanges.

III CONCENTRATIONS

A Waste outfalls contain from a few ppm up to 13% of organic contaminants.

B Surface waters contain organic contaminants in concentrations ranging from less than ppb to several ppm.

C Organic refractory substances cause serious problems in comparative trace amounts.

IV PROBLEMS

A Taste and odor are usually the first effects noticed.

B Increased chlorine and carbon demand interfere with coagulation and increase treatment costs.

C Fouling of ion exchange resins and malodors in food and beverage industries.

D Adverse effects on aquatic forms that support higher aquatic life, off-flavors in fish flesh, and direct toxic effects on fish.

E Potentially long-term toxic effects and possible carcinogenic effects on humans.

V COLLECTION, ISOLATION AND IDENTIFICATION TECHNIQUES

A Sample Collection

1 Grab samples from 1 to 5 liters are adequate in many cases.

2 The carbon adsorption method (CAM) is the most common concentration technique.

3 The megasampler, a scaled-up version of the CAM, is used in special studies where large quantities of organic materials are needed.

B Isolation

1 The carbon samples are sequentially extracted with chloroform and ethyl alcohol to desorb the organic material from the carbon.

After extraction, the excess solvent is removed, and the samples are brought to dryness to yield:

a) Carbon chloroform extract (CCE).

b) Carbon alcohol extract (CAE).

2 The CCE is separated into broad classical groups by techniques based on solubility differences.

3 Further separations are made by such techniques as adsorption, paper, and thin layer chromatography.

C Identification

1 Tentative identification is normally made using gas chromatography, paper chromatography, thin layer chromatography, and fluorescent spectroscopy.

2 Positive identification generally requires the use of infrared spectroscopy, mass spectroscopy, mass spectroscopy in conjunction with gas chromatography, and nuclear magnetic resonance (NMR); sample sizes of a few mg are required for NMR.

VI SPECIFIC COMPOUNDS DETECTED AND IDENTIFIED USING THE CAM

A These compounds have been detected and identified.

α-conidendrin; o-nitro-chlorobenzene; phenyl ether; ABS detergents; nonionic detergents; 2-ethylhexyl phthalate; dieldrin; endrin; DDT; DDE; DDD; aldrin; heptachlor; heptachlor epoxide; BHC; parathion; methyl parathion; malathion; sevin; thiodan; chlordane; telodrin; technical and γ-chlordane; bis-(2-chloroethyl) ether; bis-(2-chloroisopropyl) ether; naphthalene; tetralin; styrene; acetophenone; ethyl benzene; 2-ethyl hexane; di isobutyl carbinol; phenyl methyl carbinol; 2-methyl-5-ethyl pyridine.

B These compounds have been detected but not identified specifically.

Nitriles; amines; phenols; acids; ketones; aldehydes; alcohols.

VII TREATMENT AND REMOVAL PRACTICES

A Plant treatment procedures, such as coagulation, sedimentation and filtration, are generally not too effective.

B Chemical Treatment

1 Copper sulfate used to control algae.

2 Oxidizing agents, such as chlorine, chlorine dioxide, ozone, and potassium permanganate, are used with varying degrees of success.

3 Activated carbon treatment removes organic substances by adsorption.

C Biological Treatment

1 Natural degradation in unsaturated soils and streams.

2 Biological oxidation of organic materials both in streams and acclimated systems.

VIII SUMMARY

The problems associated with trace organic contaminants in water are becoming more apparent as our needs and usage of water increase. The continued growth of the chemical industry, our increasing population, and the public's demand for more palatable water, emphasize even more the urgency of these problems. Developments have been made in the detection of these refractory materials and in their removal from water supply sources. It is apparent, however, that further advances in the collection, identification, and removal of these pollutants are needed to insure the public of high-quality water and water resources.

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This outline was prepared by R. L. Booth, General Analysis Group, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

odor, organic compounds, organic matter, taste, water pollution, water quality

METHODS OF RECOVERING ORGANIC MATERIALS FROM SURFACE WATERS

Methods of recovering organic materials from water may be classified according to the degree of concentration required before the desired analytical procedure can be applied.

I CONCENTRATED SOLUTIONS

When the concentration is high and/or the methods are sufficiently sensitive (i.e., when only a minor degree of concentration is involved), the following methods should be considered.

A Liquid-liquid extraction usually involves water and an immiscible organic solvent. Solvents should be investigated in the series of increasing polarity:

Aliphatic hydrocarbons
Aromatic hydrocarbons
Ethers
Chlorinated compounds
    (CCl_4, $CHCl_3$, CH_2Cl_2)
Esters
Alcohols, amines, acids, etc.

Because of hydrolysis or decomposition, pH may be of critical importance; this point will be discussed at length in the discussion of Analytical Procedures. Inorganic salt concentration may also be important.

Remember the concept of the partition coefficient:

$$K = \frac{C_s}{C_w}$$

where K is the partition coefficient,

Cs is concentration in the extracting solvent

Cw is the concentration in water.

Continuous extractors must be used where the K value is not favorable to the extracting solvent. Continuous batch and counter-current extractors may be used.

Separatory funnels are available in a variety of sizes. Batch continuous extractors are commercially available in sizes up to one liter or so, and can be readily assembled in larger sizes. Continuous counter-current extractors are convenient for up to 10-20 gallons.

B Steam distillation can be used to [illegible] a sample of small amounts of [illegible] organic compounds; in this ca[illegible] distillate usually must be further [illegible] centrated by liquid-liquid extraction. The organic compound should have at least a moderate vapor pressure at 100°C and should be almost insoluble in water.

A variation of this method is simple evaporation to concentrate non-volatile organic compounds.

C Precipitants may be used: silver salts of acids, chloroplatinates or tetraphenyl boron, derivatives of amines, phenylhydrazine derivatives of ketones and aldehydes, etc.

D Ion exchange may be used to concentrate acids and bases. The only limit to the volume of water to be filtered is the amount of inorganic salts in the water; these inorganic salts usually use up the exchange capacity too rapidly to make this method practicable.

II VERY DILUTE SOLUTIONS

To concentrate extremely dilute solutions, the carbon filter is the most useful method. The organic matter is adsorbed from aqueous solution and desorbed by an organic solvent. The great advantage is the large amount of water that can be put through a small filter; the disadvantages lie in the lack of quantitative adsorption and desorption.

A The Adsorption Process

1 The adsorption process involves an equilibrium in solution.

$$\text{Adsorbed} \rightleftharpoons \text{Unadsorbed}$$

2 The Freundlich Isotherm is often useful in evaluating adsorption behavior.

$$\frac{x}{m} = kc^{\frac{1}{n}}$$

Units may be chosen for convenience, the larger the $\frac{1}{n}$, the stronger the adsorption. The constant k gives the $\frac{x}{m}$ value at unit concentration.

x = weight of adsorbed compound
m = weight of carbon
k = constant = x/m when c is unity
c = equilibrium concentration of organic compound in the liquid phase; it is measured directly.
$\frac{1}{n}$ = constant determined by the slope of the isotherm

3 General Rule: Non-polar compounds are strongly adsorbed and polar compounds weakly adsorbed from water (e.g., mineral oil is more strongly adsorbed than glycine).

4 Adsorption will depend on the type of carbon, grain size, pH of solution, polarity of adsorbate, nature of the solvent, temperature, etc. If powdered carbon is not used, the column length and contact time must be chosen so as to permit efficient adsorption.

5 Desorption is simply the reverse reaction in the adsorption equilibrium and is influenced by the same factors as adsorption. It is usually necessary to use an organic solvent for desorption with an extended period of extraction.

B Setting up the Carbon Filter

1 In the next section, is a description of the procedure used in setting up and running carbon filters in our laboratory. However, it should be understood that elaborate apparatus is not required and that excellent results can be obtained with a piece of glass tubing closed at either end by a rubber stopper in which is inserted a piece of smaller glass tubing. Such apparatus is adequate for testing small volumes (e.g., sewage or concentrated industrial wastes), although not convenient for handling thousands of gallons of water.

2 Wherever the investigation is concerned only with certain specific compounds, bench tests in small columns should be carried out in order to evaluate the performance to be expected from the filter.

III INSTALLATION OF EQUIPMENT AND COLLECTION OF CARBON FILTER SAMPLES

A The carbon filter consists of a piece of pyrex glass pipe, 3" in diameter and 18" in length. The ends are fitted with brass plates and 3/4" galvanized nipples. A stainless steel screen is fixed in a neoprene gasket at both ends.

B Presettling, Prefilter, and Backwash

River water will frequently clog the carbon filter before the desired amount of water has been sampled. It is necessary to remove sufficient turbidity to permit the required amount of water to pass through the unit. This may require a presettling tank, and a prefilter containing sand and gravel. For waters having less than 100 ppm of turbidity, a presettling tank generally is not required. Tap water, of course, requires no prefiltering and may be passed directly into the carbon filter.

C Presettling Tank

A standard hot water tank connected with the inlet at the bottom and outlet at the top, with a clean-out tap at the bottom, can serve as a presettling tank. The outlet connects to the prefilter containing sand and gravel. The hot water [illegible] should be flushed at frequent intervals to prevent large accumulations of solids. Open settling tanks can be used if care is taken to prevent long detention times and biological action. With open tanks a pump will be required to move the water through the filter.

D Sand Prefilter

The sand prefilter consists of a standard piece of steel pipe, 3" in diameter and 3 feet long, threaded at both ends and equipped with 3"×1" reducer couplings. Two disks of stainless steel screen are fitted to the inside diameter of the pipe. A simple way to prepare and hold the screen in place is to cut out a disk about the size of the outside diameter of the reducer and then push it into place so that is is below the threads. The screen is held tightly against the inside of the reducer. The pipe is packed with 6" of 1/8" gravel, 24 inches of 0.6 - 0.8 mm sand, and another 6" of 1/8" - 1/4" gravel. No free space is left in the pipe. The gravel should be packed by jarring while filling the pipe. This arrangement provides a strainer rather than a filter with a movable bed. With such an arrangement, backflushing can be done without disturbing the filter. The construction details are shown in Figure 1. The dimensions may be varied according to local conditions.

IV FILTER ARRANGEMENT

A The presettling tank, the sand prefilter, and the carbon filter should be installed at the most convenient source of raw water. If less than 15 psi pressure is available, it may be necessary to pump the water through the system. A schematic drawing of a workable system is shown in Figure 2. Exact lengths of pipes, etc., are not given since these will vary with the local situation. Both the sand and carbon filters should be connected with unions at both ends for easy removal. A typical installation is shown in Figure 2.

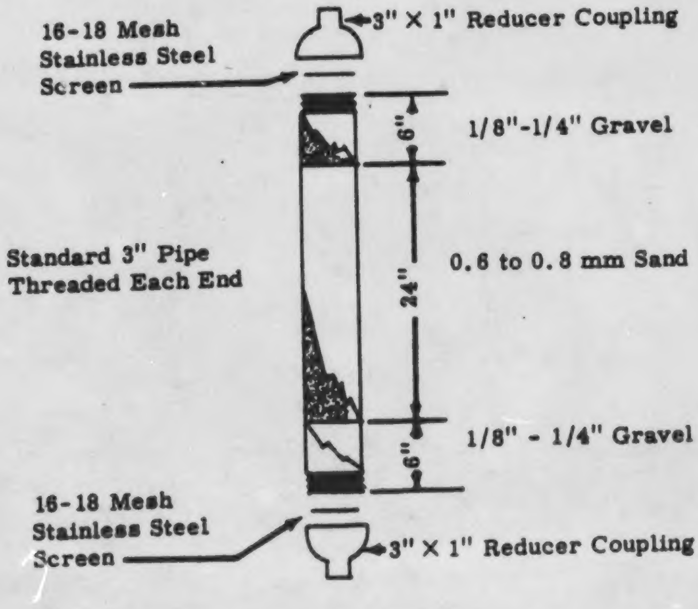

FIGURE 1 - Details of Sand Prefilter

B The raw water passes into the bottom of the sand prefilter, around into the bottom of the carbon filter. When the rate of flow through the system falls below 1/4 gpm, backwashing of the sand filter is necessary, using a high pressure source of water. A clean hose is connected to the top of the sand filter, the valve to the carbon filter is closed and the drain valve on the sand filter is opened. The sand is back-flushed until the water coming out is clear. The length of time between backwashings will vary. On the Missouri River, for example, it has been found that once every 24 hours is sufficient. After backwashing, connect the system as before and continue the sampling. Be sure to disconnect hose at top of sand filter after backwashing. A pressure gauge is inserted in the system to indicate when clogging is taking place in the carbon filter. Total pressure in the filter should not exceed 50 psi.

C A water meter located at the end of the system to prevent excessive fouling of the meter, is used to measure the volume of water samples. It is good practice to disassemble and clean the meter thoroughly after each run. A disk-type meter, 5/8"×3/4", is fairly satisfactory. A valve following the meter serves to throttle the flow, if necessary. A flow-regulating device may also be used for this purpose. The complete installation is shown in the schematic diagram in Figure 2.

D Fine carbon washes out of the filter when it is first started. A few gallons of water can be passed through the top connection and through the carbon filter drain before cutting in the meter to keep the meter free of carbon. The hose connection and drain on the carbon filter can be used to back-flush the carbon should it become clogged. This is a last resort and should only be used if absolutely necessary.

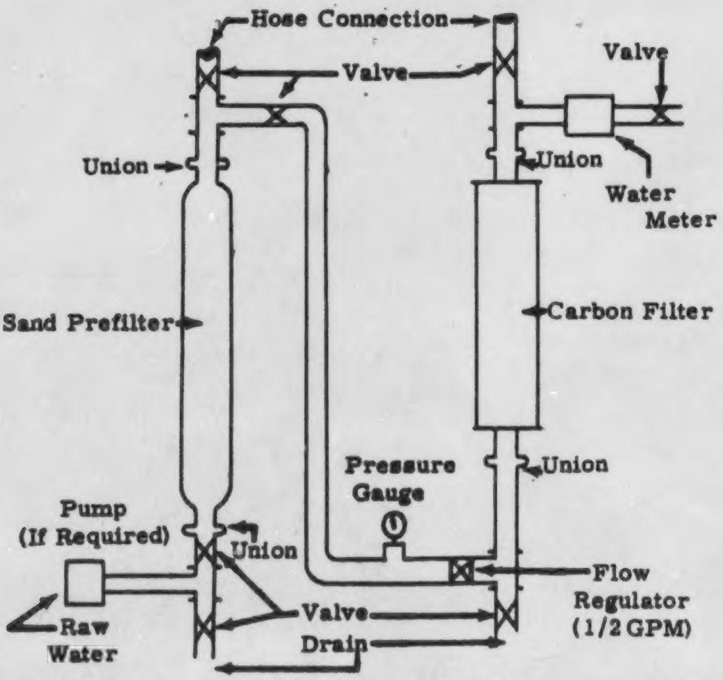

Figure 2 - Schematic Diagram of Piping Installation for Sand Prefilter and Carbon Filter

E The system outlined is not intended to remove all traces of turbidity from the water before passing through the carbon filter. Its purpose is to take out gross materials and most organisms, and to permit the required volume of water to pass through the carbon. The valve nearest the bottom of the sand filter is to be closed when it is desired to obtain a sample of raw water directly for other analyses. The drain valve is opened and, after flushing, the necessary raw water sample is collected.

F Pumping

1 If adequate pressure is not available for sampling raw water, it is necessary to pump through the filter system. It is important that the pump used should not contaminate the sample through oily packing or other sources. New pumps are oftentimes grease-coated and should be thoroughly cleaned before being put into service. If a lift of more than 12 feet is required, it will be necessary to use a jet-type pump. A model suitable for the conditions should be selected.

2 Before a new pump is put into service, it should be thoroughly flushed with hot water containing a little detergent. The pump should be operated against the minimum amount of head required to get the water through the filter. This may require by-passing of part of the flow.

G Collection of Sample

1 With fairly concentrated samples (sewage, industrial wastes, etc.), a small filter and a few gallons of water may be sufficient. Generally, for river sampling, water should be passed through a large filter at a rate of 1/4 - 1/2 gallon per minute, until up to 5000 gallons or more have been filtered. With highly turbid waters clogging may occur earlier. Although a suitable sample can be obtained sometimes with several hundred gallons of river water, it is desirable to filter a minimum of 2000 gallons if at all possible. A flow-regulating device set at 1/4 or 1/2 gpm can be placed in the system ahead of the carbon filter. A suitable unit can be obtained from Dole Valve Company, 6201 Oakton Street, Morton Grove, Illinois. These devices generally need a minimum pressure of 15 psi for proper operation.

2 Since the purpose is to get a quantitative measure of organics in water, it is very important to have accurate flow measurements. If difficulties in flow occur, this should be noted on a log sheet. Meter readings should be recorded daily on log sheets and should be designated either in GALLONS or CUBIC FEET. If a satisfactory meter can not be obtained, the flow rate for a set volume (1 or 2 liters) can be determined at regular intervals.

H Precautions

The purpose of the carbon filter is to adsorb small amounts of impurities from the water. It is important to avoid contamination of the carbon from other sources. Hence, the following should be observed.

1 New galvanized fittings are usually coated with oil or grease. The oil should be removed with a wash in kerosene followed by a detergent wash before such fittings are used for making connection to the filter.

2 Ordinary organic pipe jointing compounds should not be used. Red lead (lead oxide) mixed to a paste with water, can be used for this purpose.

3 Plastic hose is to be avoided. If rubber hose is used in any connections it should be flushed thoroughly before being connected to the filter. Copper tubing is ideal for connections.

I After the required volume of water has been run through the filter, the carbon is removed, dried, and extracted. The wet carbon is spread out in a thin layer on a metal sheet and air-dried for several days. The time needed for drying increases with the thickness of the layer. Warm air can be passed over the surface of the carbon to hasten the drying, but with the risk of driving off weakly held volatile substances.

V LABORATORY TREATMENT OF SAMPLES OBTAINED BY THE CARBON ADSORPTION TECHNIQUE: EXTRACTION PROCEDURES

The sample is collected by passing approximately 5000 gallons of water through a carbon adsorption unit. The unit is shipped to SEC, along with a daily record of the sampling activity and the carbon treated as described below.

A Preliminary Treatment

1 Records

- a Log sample in. List date received. Assign it a number.
- b Send daily record sheet to the laboratory where pertinent data is recorded on a data card (i.e., source, location, dates sampled, date received and total flow).

2 Drying

- a Remove wet carbon from the tube and dry it on copper, brass or stainless steel trays in an oven at 40°C. (n.b. Air circulated through drying cabinet should be prefiltered through carbon to prevent adsorption of foreign materials.) Drying requires about two days.
- b Store carbon samples in one gallon paint cans tightly closed.

B Solvent Extraction of Carbon

It is necessary that blanks be run on all solvents and on the carbon used for the collection of the sample.

1 Packing the extractors

- a Filter paper at bottom of soxhlet.
- b Glass wool (pre-extracted) 3" in depth to prevent carbon fines from passing into the pot. Add chloroform to wet the wool.
- c Carbon is added and packed. (n.b. Do not pack too tightly.)

2 Chloroform extraction

- a Add chloroform - about two cylinder volumes.
- b Extract for 35 hours.
- c Siphon and blow the bulk of the chloroform into the pot with air.
- d Remove the flask containing chloroform solution from the system. Concentrate to 250 ml by distillation and filter into a 300 ml Erlenmeyer flask. Evaporate to about 20 ml on a steam bath with a stream of air and transfer to a tared 5 dram vial. The remaining solvent is evaporated at room temperature without an air jet and the weight obtained.

3 Alcohol extraction

- a Remove residual chloroform from the carbon by one of the following methods.
    - 1) Blow warmed (40° C) prefiltered air upward through the carbon for about four hours.

2) Leach residual chloroform off the carbon with ethyl alcohol (95%) and distill off the chloroform alcohol mixture. Repeat until virtually pure alcohol remains.

3) Remove the carbon from the soxhlet and air dry on trays. When dry, repack into soxhlet as before. (Chloroform vapors must be disposed of.)

b Add sufficient ethanol (about two cylinder volumes) and extract for 24 hours.

c Concentrate the alcohol solution as in the case of chloroform, except that the final drying can be carried out on a steam bath with a stream of air.

The first method listed is the most satisfactory for our work here, mainly because a minimum of supervision is required and chloroform vapors are exhausted out the hoods.

C Extractable Materials

1 Calculation of concentrations

On most waters, it is convenient to compute the recovery in parts per billion (ppb) (i.e., $\mu g/l$), using the following formula.

$$\text{ppb} = \frac{\text{grams recovered} \times 10^6}{\text{gallons filtered} \times 3.785}$$

2 Infrared spectra

Infrared spectra are routinely run on both the chloroform and alcohol extracts.

D Special Applications

Other solvents may find use in performing extractions. If special applications are needed, a testing program is necessary to establish what solvents may best be used.

E Adsorption and Desorption

The effectiveness of adsorption and desorption varies for different materials and should be considered in interpretation of the results.

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Descriptors: Absorption, Adsorption, Carbon Filters, Filtration, Organic Matter, Separation Techniques, Solvent Extractions, Water Analysis

PRELIMINARY SEPARATION OF EXTRACTS

I INTRODUCTION

As a general principle, it may be stated that the more sensitive modern analytical techniques (gas and other forms of chromatography, spectrophotometry, etc.,) require fairly pure samples to begin with. Also as a general principle, it may be said that the more widely different the purification steps employed, the greater the degree of purification.

Ion exchange, dialysis, crystallization, electrophoresis, etc., are useful but cannot be discussed here. We will cover briefly only three techniques.

A Distillation

B Partition

C Adsorption

II THEORETICAL BACKGROUND

A Distillation

1 Raoult's Law states that the partial pressure of A(P_A) in an ideal mixture of volatile solvents is

P_A = mole fraction of A in liquid × vapor pressure of pure A at the temperature of the system.

The total vapor pressure (which, of course, sets the boiling point) is then $P_A + P_B + \ldots$. This is the basis for studies of distillation. The question of non-ideal solutions is too complicated to discuss. Most physical chemistry texts go into it at length.

2 Partially miscible liquids are a special case, often discussed as "steam distillation" although co-distillation is a better term. Quoting from Glasstone, "As long as the two layers are present, irrespective of their relative amounts, the total vapor pressure remains constant and the system boils at a definite temperature."

Besides the familiar case of steam distillation, non-polar organics can be co-distilled with polar liquids, e.g., insecticides with glycerol and polar compounds with non-polar solvents (i.e., mineral oil). The co-distillation can be done with superheated solvents, under vacuum. Where trace quantities of one component are involved, there may not be a two-phase system present and the solvent vapor serves only as a carrier gas. Here the solute can usually be recovered easily, however, so the method is worth consideration regardless of the mechanism.

In practical work, the three important points are the stability of the sample, its volatility, and the ease of recovery from the distillate.

3 Distillation from acid and basic solutions may also be useful. The principle behind this will be discussed in the solubility separation method.

B Partition

Partition is the principle utilized in liquid-liquid extraction, paper chromatography and gas chromatography. The fundamental law is the Distribution Law: "A dissolved substance distributes itself between the layers of a two-layer system so that at constant temperature the ratio of the concentrations is also constant". The Law is properly applied only to dilute solutions, but a first approximation to the distribution ratio can be obtained from the ratio of solubilities.

$$K = \frac{C_s}{C_w} \qquad K = \frac{S_s}{S_w}$$

K is a true constant but apparent discrepancies occur where the solute has different molecular weights in the two phases. Benzoic acid, for instance, is largely ϕCOOH plus some benzoate ion in water; in benzene it is the dimer $(\phi COOH)_2$. K then applies only to the species common to both layers, ϕCOOH, and ignores the dimer.

Most texts on qualitative organic analysis contain a great deal of practical information on solubilities, while the usual undergraduate physical chemistry texts cover the essentials of the theory.

C Adsorption

There are two kinds of adsorption to be considered: physical (van der Waals) adsorption, and chemisorption. The theory of adsorption from solution is not as well understood as adsorption of gases but we can make a few comments.

Physical adsorption depends on the relatively weak van der Waals forces due to electrostatic attraction of dipoles, it being assumed that non-polar molecules act as oscillating dipoles; i.e., the nuclei of atoms and molecules form oscillating dipoles with the electrons. Since the bonds are weak, adsorption can be made reversible under proper conditions.

Chemisorption is thought of as actual chemical reaction, and the resulting bonds are much more difficult to break. Losses due to "irreversible adsorption" may therefore appear; this is particularly true of carbon, less so of silica and alumina.

III SOLUBILITY SEPARATION OF EXTRACTS

In the next section, we will describe a laboratory procedure which we have found very useful in handling carbon filter extracts. The extracts are split into acid, basic, and neutral fractions, and the neutral fraction is further separated by adsorption chromatography. This procedure has been extremely useful where the fractions are weighed and infrared spectra made. However, where volatile compounds are to be looked for by gas chromatography, it is almost imperative that a steam distillation step be included. Otherwise the mass of heavy, probably polymeric, material present causes much interference.

Really good clean-up methods are likely to be highly specific and need to be "tailor-made" to suit the situation. Since this lecture is concerned only with the "preliminary" purification, we have omitted a discussion of precise analytical partition and adsorption columns (thin-layer, ion exchange, paper and gas chromatography, gradient elution methods, etc.) Such methods constitute a second and higher degree of purification, but ordinarily require that they be preceded by a "rougher" primary purification.

IV NOTE ON LOSSES OF VOLATILE COMPOUNDS

It may not be generally recognized, but it is quite true that heavy losses of even moderately volatile compounds occur if one tries to evaporate off all of the solvent when preparing to weigh an extract. We have had losses of over 50% in attempting to carry out what we thought were very careful evaporations of ether from 10 to 20 mg amounts of phenols.

For this reason, we suggest that no extract or fraction thereof be evaporated down for weighing unless it is known to be completely non-volatile. This precaution becomes more necessary as the purity of the sample increases, because non-volatile impurities act as "solvents" to reduce the amount of loss (according to Raoult's Law).

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1 Maron and Prutton. Principles of Physical Chemistry (4th Edition). The Macmillan Co. 1965.

2 Moore. Physical Chemistry (3rd Ed.) Prentice Hall, Inc. 1965.

3 Glasstone and Lewis. Elements of Physical Chemistry. D. van Nostrand Co. 1960.

This outline was prepared by R. H Burtschell, Chemist, formerly with Waste Identification and Analysis Program, Advanced Waste Treatment Research Laboratory, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Absortpion, Adsorption, Distillation, Organic Matter, Separation Techniques, Water Analysis

LABORATORY PROCEDURES FOR THE PRELIMINARY SEPARATION OF EXTRACTS

I STEAM DISTILLATION OF EXTRACTS

Steam distillation need be only a very simple procedure. Put a gram or two of crude or partially purified sample in a small flask containing 50 - 100 ml of distilled water. Set up for ordinary distillation and boil off most of the water. Set up for ordinary distillation and boil off most of the water. If necessary, add more water to the flask and continue distilling until the distillate has no tract of cloudiness or a second phase as it drips from the condenser. Transfer the distillate to a separatory funnel, extract with a suitable solvent, and concentrate to a convenient volume. As the product is relatively volatile, do not try to evaporate to dryness if it can be avoided. The non-volatile residue in the distillation flask can also be recovered if necessary.

If it is desired to recover acids (e.g., phenols in the 4-AAP colorimetric method), make the solution acid before distilling; the distillate will then contain only neutral and acidic substances. The neutrals can then be separated during the extraction step. Basic materials remaining in the still pot, e.g., aniline, can then be recovered by making basic and distilling over a second fraction.

II SOLUBILITY SEPARATION OF EXTRACTS

A The organic contaminants from water as extracted from carbon or concentrated by liquid extraction ordinarily form an exceedingly complex mixture for which there is no one satisfactory separation procedure. If the analysis is directed towards a single component, the procedure may be designed for that purpose; otherwise a useful and generally applicable preliminary separation may be made on the basis of relative acidities.

B By extracting an ether solution of the sample with water, then with dilute hydrochloric acid, then sodium bicarbonate, and finally sodium hydroxide, a separation into water soluble, basic, strongly acidic, weakly acidic, and neutral fractions may be made. The portion insoluble in ether may also be recovered as an additional fraction. This method is obviously not suited to very volatile substances, nor to further separation of the water soluble fraction, nor to substances unstable in the presence of water or dilute acid or base; in addition, it must be remembered that substances whose partition coefficients are not extremely unfavorable to water may be present in several fractions in varying amounts.

C This procedure constitutes one of the best preliminary steps in analyzing any unknown sample and may often profitably be either preceded or followed by steam distillation (at various pH's) fractional crystallization, etc. Simple micro qualitative tests for nitrogen, halogens, sulfur, phosphorus, etc., are also often useful; for a more extended discussion, see the texts mentioned in the bibliography.

General

The sample should be substantially free of solvent. The amount may vary considerably but one-half gram is a very convenient amount; as little as 100 mg or even less may be used, but in this case there may be large percentage errors. Ethyl ether has proven to be an excellent solvent although often not all the sample will dissolve in it; benzene or chloroform may be used although these solvents are more likely to cause troublesome emulsions.

III LABORATORY DIRECTIONS

A Solubility Separation Procedure

Solution in Ether

1 Weigh previously dried sample contained in a tared 50 ml beaker (approximately 0.5 gm preferred). Also, weigh a 125 ml flask for the Water Solubles (WS).

2 Dissolve the sample with a small amount of methanol (about 1 ml). Add the methanol dropwise and stir with a rigid wire until the sample reaches a syrupy consistency.

3 Add 30 ml ether to the sample. Not all will dissolve. Collect the Ether Insolubles (EI) on a sintered glass funnel with suction and set aside for paragraph 4. Pour the contents of the suction flask into a 125 ml separatory funnel equipped with a teflon stopcock. (Stopcock grease will contaminate the sample.)

Ether Insolubles (EI)

4 Dissolve "EI" previously collected on the sintered glass funnel with methanol. Wash funnel once with a small volume of chloroform and collect in the suction flask. Transfer filtrate to the original tared beaker with methanol. Evaporate to dryness on a steam bath and record weight for Ether Insolubles.

Separation of the Water Solubles (WS)

5 Shake the ether solution$^{(3)}$ three times with 15 ml portions of distilled water. Drain the water layers into the weighed "WS" flask after each shaking. Evaporate the Water Soluble fraction to dryness on a steam bath with a jet of clean, dry air and record weight for Water Solubles.

Separation of Amine Fraction (B)

6 Mark flask "HCl". Shake ether solution three times with 15 ml portions of 5% HCl and drain aqueous layers into HCl flask after each shaking. Make this aqueous solution strongly basic by carefully adding NaOH pellets (about 30) or 25% NaOH. The solution becomes darker at this point. Set aside for back-extraction.

Separation of Strong Acid Fraction (SA)

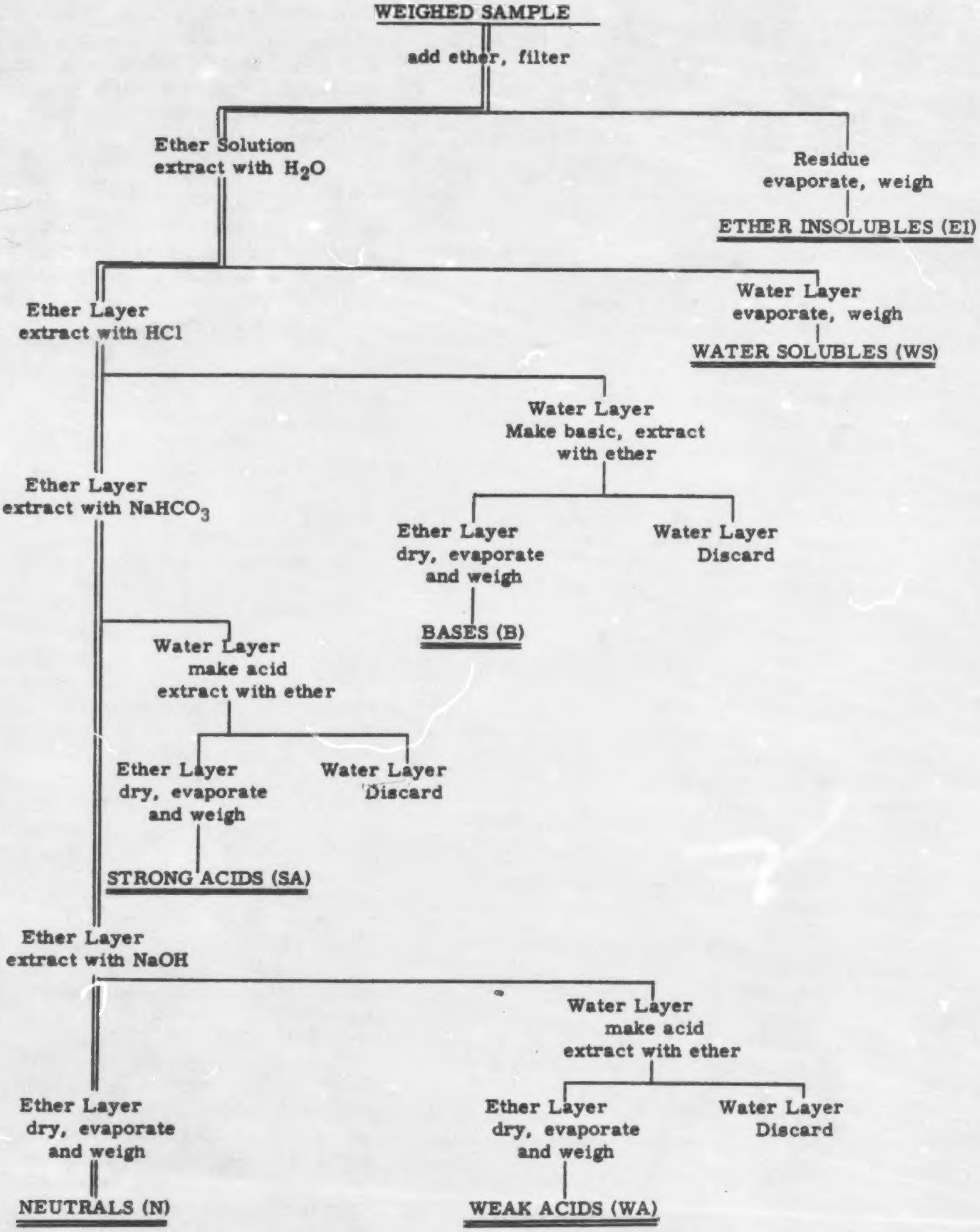
7 Restore, original ether volume to 30 ml, if necessary. Mark flask "$NaHCO_3$". Shake three times with 15 ml portions of 5% $NaHCO_3$ and drain aqueous layers into $NaHCO_3$ flask after each shaking. Acidify aqueous solution in flask by adding sufficient concentrated HCl (dropwise because of liberation of CO_2) until strongly acid to litmus paper. About 4 ml is sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

Separation of Weak Acid Fraction (WA)

8 Restore original ether volume to 30 ml, if necessary. Mark flask "NaOH". Shake three times with 15 ml portions of 5% NaOH and drain aqueous layers into "NaOH" flask after each shaking. Wash once with 15 ml distilled water and drain this into "NaOH" flask.

CAUTION: Tilt separatory funnel gently a few times, instead of shaking the first portion, as emulsions often occur.

If a small amount of the water wash remains emulsified, after draining, add several drops of saturated Na_2SO_4 solution and shake. This may help to break


WEIGHED SAMPLE
add ether, filter
Ether Solution
extract with H_2O
Residue
evaporate, weigh
ETHER INSOLUBLES (EI)
Ether Layer
extract with HCl
Water Layer
evaporate, weigh
WATER SOLUBLES (WS)
Water Layer
Make basic, extract
with ether
Ether Layer
extract with $NaHCO_3$
Ether Layer
dry, evaporate
and weigh
Water Layer
Discard
BASES (B)
Water Layer
make acid
extract with ether
Ether Layer
dry, evaporate
and weigh
Water Layer
Discard
STRONG ACIDS (SA)
Ether Layer
extract with NaOH
Water Layer
make acid
extract with ether
Ether Layer
dry, evaporate
and weigh
Ether Layer
dry, evaporate
and weigh
Water Layer
Discard
NEUTRALS (N)
WEAK ACIDS (WA)

the emulsion. Acidify aqueous solution in flask with concentrated HCl until strongly acid to litmus paper. About 8 ml sufficient. The solution becomes cloudy at this point. Set aside for back-extraction.

Neutral Fraction (N)

9 Mark flask "N". Pour the ether layer remaining in the separatory funnel into the flask "N". Add about 10 gm of anhydrous sodium sulfate, cap with aluminum foil and allow to stand overnight to dry the ether solution.

B Back Extraction

Strong Acids (SA)

10 Mark flask "SA". Transfer $NaHCO_3$ extract from step 7 to separatory funnel and shake several times, carefully, relieving pressure caused by evolution of CO_2. Shake the $NaHCO_3$ extract, previously made acid, with a 15 ml portion of ether. Caution: Release CO_2 during first shaking repeatedly to avoid pressure build-up.

Drain the aqueous layer into the $NaHCO_3$ flask. Pour the ether layer into the "SA" flask. Return aqueous layer to separatory funnel and repeat extraction two more times with 15 ml portions of ether.

After all the ether layers have been collected in the "SA" flask, add about 10 gms anhydrous sodium sulfate, cap with foil and allow to stand overnight to dry the ether solution. Discard the aqueous $NaHCO_3$ portion which should be almost colorless.

Weak Acids (WA)

11 Mark flask "WA". Shake the NaOH extract, previously made acid, three times with 15 ml portions of ether and collect in flask "WA" as in step 10. Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous NaOH portion which should be almost colorless.

Bases (B)

12 Mark flask "B". Shake the HCl extract, previously made basic, three times with 15 ml portions of ether and collect in flask "B". Add sodium sulfate to dry, cap with foil and allow to stand. Discard aqueous HCl portion.

C Transfer

13 Weigh four flasks and mark them "N", "WA", "SA", and "B". Record weights of each. Transfer the corresponding dried ether solutions from the Na_2SO_4 into the weighed flasks by filtering through filter paper.

14 Evaporate the ether from the above fractions with the aid of clean, dry circulating air and gentle heat on a steam bath. The ether solutions should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components. Record the weight of the dried fractions.

D Chromatographic Separation

15 Weigh a 10 ml beaker and record weight. Dissolve the Neutrals in about 5 ml anhydrous ether, transfer most of the Neutrals to the beaker and place the remaining few drops in a vial for infrared analysis. Wash the (N) flask with ether and pour washings into vial also. Dry the Neutrals contained in the beaker by spontaneous evaporation and record weight.

16 Weigh a set of 125 ml Erlenmeyer flasks and mark them Aliphatics, Aromatics and Oxys. Record weights of each.

17 Fill the chromatographic column with 4 1/2" of silica gel and tap down to about 4". Place the Aliphatics flask under the column. Add about 20 ml of iso-octane to wet the column.

Aliphatics

18 Before the iso-octane reaches the level of the silica gel, add the Neutrals which have been previously adsorbed onto a small amount of silica gel by stirring in the beaker with a rigid wire. Elute with 85 ml iso-octane. Rinse the beaker with a small portion and add to the column first. Allow this volume to enter silica gel before adding the remainder of the iso-octane. Collect the eluent in the Aliphatics flask, evaporate to dryness with the aid of gentle heat and circulating air.

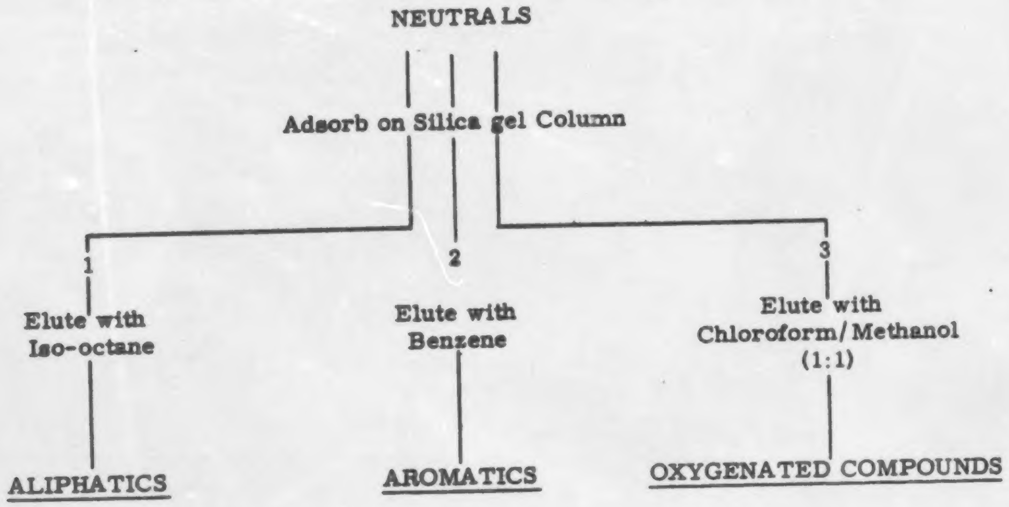
Aromatics

19 Place the Aromatics flask under the column. Elute column with 85 ml of Benzene-rinsing the beaker with a small portion and adding immediately as the previous eluent reaches the level of the silica gel. Allow this volume to enter silica gel before adding remainder of benzene. Evaporate with the aid of gentle heat and circulating air. The Aromatics fraction should be removed from the air and heat before totally dry and allowed to dry spontaneously to prevent loss of volatile components.

Oxys

20 Place the Oxys flask under the column. Elute with 85 ml of a 1:1 methanol chloroform mixture - rinsing the beaker with a small portion and proceed as before. Evaporate to dryness with the aid of gentle heat and circulating air.

CHROMATOGRAPHIC SEPARATION OF NEUTRALS



21 Record weights of the dried Aliphatics, Aromatics and Oxys.

NOTE: (All chromatographic solvents should be redistilled before use)

REFERENCES

1 Shriner, Fuson and Curtin. The Systematic Identification of Organic Compounds. 4th ed. John Wiley & Sons. New York. 1956.

2 Cheronis and Entrikin. Semimicro Qualitative Organic Analysis. Thomas Y. Crowell Co. New York. 1947.

3 Cheronis. Micro and Semimicro Methods. Vol. VI of the series Techniques of Organic Chemistry. Arnold Weissberger, Ed. Interscience. New York. 1954.

4 Schneider. Qualitative Organic Microanalysis. John Wiley & Sons. New York. 1946.

These books deal principally with chemical methods of identification; Shriner, Fuson and Curtin and Cheronis and Entrikin are recommended for those new to the field. For instrumental analysis and quantitative work, see standard works in those fields.

This outline was prepared by R. H. Burtschell, Chemist, formerly with AWTL, NERC, Cincinnati, OH and J. J. Lichtenberg, Pesticides Identification Group Leader, AQCL, NERC, EPA, Cincinnati, OH 45268.

Descriptors: Laboratory Tests, Organic Matter, Separation Techniques, Solvent Extractions, Water Analysis

THIN-LAYER CHROMATOGRAPHY

I INTRODUCTION

A Historical

1 Adsorption chromatography was discovered by Tswett in 1903. Tswett found that plant pigments (e.g., chlorophylls) can be separated by filtering a solution of them in petroleum ether through a column of calcium carbonate. He noticed that yellow and green zones were formed in the column.

2 Adsorption chromatography was introduced in 1931 when the method was used for the preparative chemistry of the polyene pigments, for example the separation of carotene into its components.

3 Partition chromatography for hydrophilic compounds was introduced in 1941.

4 Thin-layer chromatography was introduced by Stahl in 1956. He described an ingenious and practicable device for preparing layers of silica gel about 250 microns thick with a plaster of paris binder.

5 Thin-layer chromatography has proven useful for the quantitative estimation of pesticides and for the "clean-up" of sample aliquots prior to the estimation of their components by gas chromatography.

B Theory

1 Thin-layer chromatography is an analytical technique which has proven useful for the separation of components in a given system and to evaluate the components in that system.

2 All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type (liquid or gas) which is in equilibrium with a stationary phase. When the stationary phase is a solid and the forces acting between it and the mixture are adsorptive in nature, the technique is called adsorption chromatography.

3 When the stationary phase is a simple liquid or a liquid held on some type of support, the chromatography is considered to be partition chromatography.

4 Diagnostic or qualitative thin-layer chromatography is used to determine the number of components in a system.

5 Preparative thin-layer chromatography is used to separate a mixture into its components in such a way that reasonable amounts can be isolated and studied.

6 Quantitative thin-layer chromatography is used to measure the amount of the components present. This is done by estimating the density of the spots as compared to standard spots.

II DEFINITION OF TERMS

A Adsorbent. The finely divided powder which makes up the stationary phase in adsorption chromatography, or holds the stationary liquid in partition chromatography.

B Layer. The thin layer of adsorbent bound or unbound, deposited on a glass plate.

C Spotting. The application of the substance to the thin layer adsorbent.

D Development. The passing of a liquid through the layer to effect a separation.

E Developer. The liquid used for the development.

F Eluent. The complete removal of a substance from the adsorbent layer.

G **Visualization.** The rendering visible of the results of a developed chromatogram.

H **Ratio facter "R_f".** The distance traveled by a given substance divided by the distance traveled by the solvent front. Both measured as the origin.

III ADSORBENTS

A Silicic Acid or Silica Gel

Chemically identical, but the gel is prepared in such a manner as to enhance its adsorptive power.

B Aluminum Oxide or Alumina

C Diatomaceous Earth

D Powdered Cellulose

E Essentially any substance which has desirable adsorptive and chemical characteristics can be successfully used in thin-layer chromatography.

IV PREPARATION OF PLATES

A Methods

1 Spreading or spraying of slurries

2 Dipping in a slurry

B Equipment

1 Kirchner type

2 Stahl type

3 Microchromatoplates

C Layer thickness

1 For qualitative analysis thin layers (250 microns) are used.

2 For preparative thin layer thicker layers may be used.

D Moisture Content

1 Trace quantities of water greatly reduce the activity of various adsorbents.

2 Normally plates are activated by drying at 110°C for one hour and then stored in a desiccator.

V TECHNIQUE

A Spotting the Plate

1 The activity of the layer is affected by humidity and temperature.

2 The sample is applied so that the direction of the development is opposite to the direction in which the layer has been applied.

3 Use of spotting template

4 Use of micro-pipette and micro-liter syringe

5 Size of sample spots

6 Use of standards

B Solvent System

1 Investigation of single and multi-component systems

2 Carbon tetrachloride, hexane, and n-heptane as solvents for separation of pesticides

C Development of the Plate

1 Development tank

2 The plate is placed in a glass tank containing the solvent. When the solvent front reaches the upper reference line (10 cm) the plate is removed and the solvent is allowed to evaporate.

VI VISUALIZATION

A Rhodamine B

1 Spray reagents - Rhodamine B, spirit soluble, 0.1 mg per ml in ethanol

2 Method of spraying

3 Advantages of Rhodamine B

  a The pesticide is not destroyed.

  b The exact position of the pesticide can be determined.

  c The use of a selective solvent permits the pesticide to be eluted from the adsorbent while the Rhodamine B is retained.

B Impregnation of Plates with $AgNO_3$

1 Increased sensitivity for quantitative estimation

2 Elimination of spraying

C Exposure to UV Light

1 Pesticides appear as quenched areas in the fluorescent background.

2 Recovery studies using Rhodamine B spray are comparable to those using silver nitrate spray. The following pesticides may be seen at the 10 µg level under UV light after Rhodamine B spray:

endrin
dieldrin
DDT
aldrin
lindane
chlordane
parathion
methyl parathion
heptachlor
heptachlor epoxide
methoxychlor
toxaphene
DDE
ovex
tedion

D Suggested systems for adsorption chromatography are given in Table 1.

E Recovery

1 Elution of pesticides from the thin layer with a mixture of ethyl and petroleum ethers.

2 Recovery zones as shown in Figure 1.

3 Silica gel collection assembly - Figure 2.

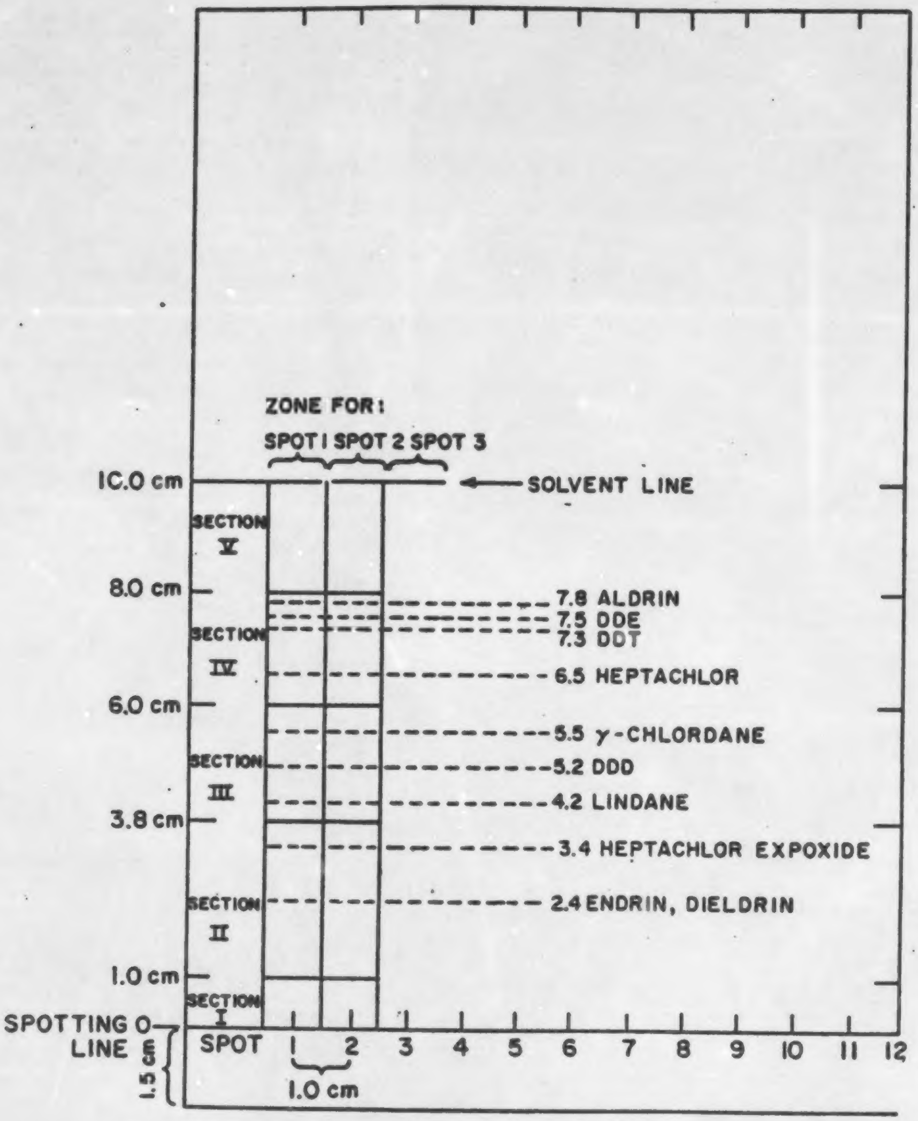
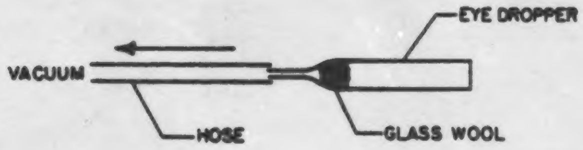

ZONE FOR:
SPOT 1 SPOT 2 SPOT 3
10.0 cm
SOLVENT LINE
SECTION V
8.0 cm
7.8 ALDRIN
7.5 DDE
7.3 DDT
SECTION IV
6.5 HEPTACHLOR
6.0 cm
5.5 γ-CHLORDANE
SECTION III
5.2 DDD
4.2 LINDANE
3.8 cm
3.4 HEPTACHLOR EXPOXIDE
SECTION II
2.4 ENDRIN, DIELDRIN
1.0 cm
SECTION I
SPOTTING LINE
0
1.5 cm
SPOT
1
2
3
4
5
6
7
8
9
10
11
12
1.0 cm

Figure 1. Diagram of Designation of Sections in the Cleanup and Separation of CCE-Aromatics on Silica Gel Layers.


EYE DROPPER
VACUUM
HOSE
GLASS WOOL

SILICA GEL COLLECTION ASSEMBLY

FIGURE 2

TABLE 1

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
1. Long-chain (over eight carbons) aliphatic ketones	silica gel G	a. benzene-EtOEt mixtures b. toluene-EtOEt mixtures c. pet. ether-EtOEt mixtures	phosphomolybdic acid (2, 4-DNPH)
2. Aliphatic and aromatic aldehydes and ketones	aluminum oxide	a. benzene b. benzene-EtOH (98:2) (95:5) (90:10) c. $CHCl_3$ d. EtOEt e. pet. ether- benzene (1:1)	2, 4-DNPH
3. Aromatic aldehydes and ketones	silica gel G	hexane-EtOAc (4:1) (3:2)	(U.V. on phosphors)
4. Vanillin and other substituted benzaldehydes	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) c. $CHCl_3$-EtOAc (98:2) d. decalin-CH_2Cl_2-MeOH (5:4:1)	(U.V. on phosphors)
5. 2, 4-Dinitrophenylhydrazones of aldehydes	silica gel G	a. benzene-pet. ether (3:1) for aliphatic b. benzene-EtOAc (95:5) for aromatic	colored
6. Miscellaneous alkaloids	silica gel G	a. $CHCl_3$-acetone-diethylamine (5:4:1) b. $CHCl_3$-diethylamine (9:1) c. cyclohexane-$CHCl_3$-diethylamine (5:4:1) d. cyclohexane-diethylamine (9:1) e. benzene-EtOAc-diethylamine (7:2:1)	(Dragendorff's Reagent)
	aluminum oxide	f. $CHCl_3$ g. cyclohexane-$CHCl_3$ (3:7) plus 0.05% diethylamine	
7. Alkaloids and barbiturates in toxicology	silica gel G	a. MeOH b. $CHCl_3$-EtOEt (85:15)	(Dragendorff's Reagent)
8. Strong-base amines	unbound aluminum oxide	a. acetone-heptane (1:1) b. $CHCl_3/NH_3$ (sat. at 22°)-EtOH (96%) (30:1)	I_2 vapor-U.V. (Dragendorff's Reagent)
9. Sugar acetates and inositol acetates	silica gel G	benzene with 2-10% MeOH	H_2O

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
10. Aldose 2,4-DNPH's	a. aluminum oxide G b. silica gel G	a. toluene-EtOAc (1:1) b. toluene-EtOAc (3:1) (1:1)	a. colored compounds b. NaOH
11. Cardiac glycosides	silica gel G	a. $CHCl_3$-MeOH (9:1) b. $CHCl_3$-acetone (65:35)	H_2SO_4 and heat
12. Dicarboxylic acids	silica gel G	a. benzene-MeOH-HOAc (45:8:4) b. benzene-dioxane-HOAc (90:25:4)	bromphenol blue acidified with citric acid
13. p-Hydroxybenzoic acid esters	silica gel G	pentane-HOAc (88:12)	(U.V. on phosphors)
14. Sulfonamides	silica gel G	$CHCl_3$-EtOH- heptane (1:1:[illegible])	p-dimethylaminobenzaldehyde, acidified (U.V. on phosphors)
15. Food dyes	silica gel G	a. $CHCl_3$-acetic anhydride (75:2) b. benzene c. methyl ethyl ketone-HOAc-MeOH (40:5:5)	$SbCl_3$ in $CHCl_3$
16. Misc. essential oils	silica gel G	benzene-$CHCl_3$ (1:1)	$SbCl_3$ in $CHCl_3$
17. Coumarins	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2)	(U.V. on phosphors)
18. Alkali metals-Na^{1+}, Li^{1+}, K^{1+}, Mg^{2+}	purified silica gel G	EtOH-HOAc (100:1)	acid violet (1.5% aqueous soln.)
19. 24 ferrocene	silica gel G	a. benzene b. benzene-EtOH (30:1) (15:1) c. propylene glycol-MeOH (1:1) d. propylene glycol-chlorobenzene-MeOH (1:1:1)	(U.V. on phosphors)
20. Misc. lipids	silica gel G	a. EtOEt b. isopropyl ether c. isopropyl ether-HOAc (98.5:1.5)	(H_2SO_4 and heat)
21. Fatty acid methyl esters	silica gel G	hexane-EtOEt mixtures (up to 30% EtOEt)	a. I_2 b. H_2SO_4 and heat
22. Glycerides	silica gel G	a. Skellysolve F-EtOEt (70:30)	H_2SO_4 and heat

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
		b. (10:90) for monoglycerides c. (70:30) for diglycerides d. (90:10) for triglycerides e. (60:40) (35:65) (85:15)	
23. Long-chain aliphatic alcohols	silica gel G	a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90) (70:30)	H_2SO_4 and heat
24. Phenols	a. starch-bound silicic acid kieselguhr (1:1)	a. xylene b. xylene-$CHCl_3$ (3:1) (1:1) (1:3) c. $CHCl_3$	(U.V. on phosphors)
25. Phenols and phenolic acids	starch-bound silicic acid with phosphors	a. Skellysolve B- EtOEt (3:7) b. Skellysolve B- EtOAc (1:3) c. Skellysolve B-acetone (3:1)	(U.V. on phosphors)
26. Phenols	plaster-of-Paris bound silica gel	a. hexane-EtOAc (4:1) (3:2) b. benzene-EtOEt (4:1) c. benzene	(U.V. on phosphors)
27. 3,5-Dinitrobenzoates of alcohols and phenols	silica gel G	a. benzene-pet. ether (1:1) b. hexane-EtOAc (85:15) (75:25) c. toluene-EtOAc (90:10)	colored compounds
28. Steroids	silica gel G	a. benzene b. benzene-EtOAc (9:1 (2:1) c. cyclohexane-EtOAc (9:1) (19:1) d. 1,2-dichloroethane	$SbCl_3$ in $CHCl_3$
29. 19-Nor-steroids	silica gel G	EtOAc-cyclohexane mixtures	$SbCl_3$ in $CHCl_3$
30. Blood cholesterol and cholesterol esters	silica gel G	a. benzene b. benzene-EtOAc (9:1) c. 1,2-dichloroethane d. $CHCl_3$	$SbCl_3$ in $CHCl_3$
31. Triterpenoids	silica gel G	a. isopropyl ether-acetone (5:2) (19:1) b. isopropyl ether c. cyclohexane d. benzene e. CH_2Cl_2	(H_2SO_4 and heat)

TABLE 1 (continued)

SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

	Compounds	Adsorbent	Developer	Visualization
32.	Carotenes and fat-soluble vitamins, A, D, E, K	unbound aluminum oxide	a. MeOH b. CCl_4 c. xylene	H_2SO_4 and heat
33.	Thiophene derivatives	a. silica gel G b. aluminum oxide G	a. benzene-$CHCl_3$ (9:1) b. MeOH c. pet. ether	(U.V. on phosphors)
34.	Plasticizers (phthalates, phosphates and other esters)	silica gel G with phosphors	a. isooctane-EtOAc (90:10) b. benzene-EtOAc (95:5) c. butyl ether - hexane (80:20)	a. (U.V. on phosphors) b. I_2

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This outline was prepared by P.F. Hallbach, Chemist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

Descriptors: Adsorbents, Chromatography, Organic Matter, Separation Techniques, Water Analysis

INTRODUCTION TO GAS-LIQUID CHROMATOGRAPHY

Part I

I INTRODUCTION

A Definition

Gas-liquid chromatography is an analytical method for the separation and identification of a mixture of volatile (usually organic) components in a sample. As with any chromatographic technique the column consists of two phases, the immobile or stationary phase (a liquid on an inert solid support), and the mobile phase (an inert gas). The column functions to separate the sample components because they have varying vapor pressures and affinities for the stationary phase. In many ways the column behavior resembles that of fractional distillation. The partition which occurs between the mobile and immobile phases will thus cause the components to proceed through the column at varying rates. The separation is recorded and quantitated by the detector system.

B Advantages

1 GLC can be used to separate compounds of similar boiling points which cannot easily be separated by distillation. (See Table 1)

2 GLC can be extremely sensitive; for example, using the electron capture detector it is possible to "see" picogram (10^{-12}) quantities.

Table 1. SEPARATIONS BY GLC

Compounds	Reference
3-Methylcyclohexene (B.P. 104° C) and 4-Methylcyclohexan (B.P. 103° C)	Aerograph Research Notes (Spring 1964)
Cyclohexane (B.P. 80.8° C) and Benzene (B.P. 80.2° C)	Chromosorb Newsletter (FF-104)

C Disadvantages

1 Due to the extreme sensitivity possible it is often necessary to apply extensive cleanup techniques.

2 The many variables of the technique require a skilled analyst.

II COMPONENTS OF A GAS CHROMATOGRAPH (See Figure 1)

A Gas Supply

The mobile phase (carrier gas) transports the sample components through the column into the detector. The type of gas used varies with the detector.(See Table 2)

B Injector

Liquid samples are manually introduced into the heated injector block through a rubber septum by means of a syringe. Automatic liquid injectors as well as injection systems for solid and gaseous samples are commercially available.

C Column

The vaporized sample enters the column which can be glass or metal and of varying length (1' - 20') and diameter (1/8" - 1/4"). The column is packed with the stationary (immobile) phase and contained within a constant temperature oven.

1 Solid support

The solid support should have a large surface area yet be inert so that active sites will not cause adsorption of sample components. Diatomaceous earths, teflon and glass beads have been used. (See Table 3)

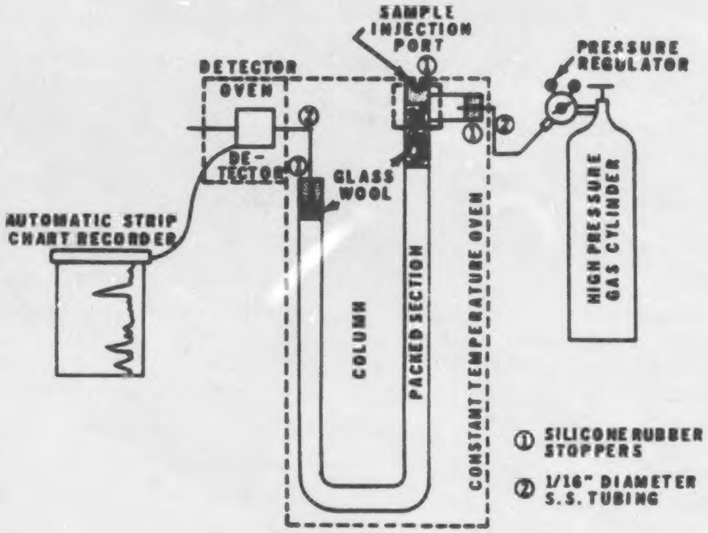
SAMPLE INJECTION PORT
PRESSURE REGULATOR
DETECTOR OVEN
DE-TECTOR
GLASS WOOL
AUTOMATIC STRIP CHART RECORDER
COLUMN
PACKED SECTION
CONSTANT TEMPERATURE OVEN
HIGH PRESSURE GAS CYLINDER
① SILICONE RUBBER STOPPERS
② 1/16" DIAMETER S.S. TUBING

Figure 1. COMPONENTS OF A GAS CHROMATOGRAPH*

Table 2. CARRIER GASES

Detector	Carrier gas
Thermal conductivity	Helium (Purified, Grade A)
Microcoulometric	Helium (Purified, Grade A)
Flame ionization	Hydrogen (Purified)
Electron capture	Nitrogen or a mixture of 95% argon and 5% methane (Purified)

Table 3. SOLID SUPPORTS

Support	Surface area (m^2/gm)
Chromosorb P (Diatomaceous Earth)	4.8
Chromosorb W (Diatomaceous Earth)	1.2
Chromosorb G (Diatomaceous Earth)	0.5
Chromosorb T (Teflon)	7.0 - 8.0

2 Stationary liquid

The separation and partition occurring in the column is directly affected by the choice of stationary liquid. For example, in the separation of benzene (B.P. 80.1°C) and cyclohexane (B.P. 80.8°C), the choice of a non-polar phase such as hexadecane results in benzene preceding cyclohexane off the column. However, if a more polar phase such as benzylbiphenyl is chosen cyclohexane precedes benzene. Table 4 shows some typical stationary liquids and their uses. (NOTE: One requirement for any liquid is that it have a high boiling point so that it will not boil off the column)

D Detector

The detector or brain of the gas chromatograph senses and measures the quantity of sample component coming off the column. The detector should be maintained at a temperature higher than the column so that condensation does not occur in the detector block. Several types of detectors are in use today.

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*Reproduced (with permission) from Chemistry. (37:11, p. 13. November 1964).

Table 4. STATIONARY LIQUIDS

Stationary liquid	Used to separate
Silicone oils QF-1, Dow Corning 200, and Dow 11, OV-1, OV-3, OV-17, OV-Z10, OV-225	Chlorinated hydrocarbons pesticides
Silicone oil SE-30	Homologous series of n-alkanes
Benzyl-Cyanide-Silver Nitrate	Homologous series of olefins
Polyethylene Glycol	Amines
Cyano Silicone	Steroids

1 Thermal conductivity

This detector consists of a Wheatstone bridge two arms of which are thermal conductivity cells each containing a small heated element. When only carrier gas is flowing through both the sample cell and reference cell, the resistance of the heated element is constant in both cells. The bridge remains balanced and baseline is recorded. However, when carrier gas plus sample component enter the sample cell, the thermal conductivity in that cell changes thus also producing a change in the resistance of the heated element. The bridge becomes unbalanced and a peak is recorded. The main disadvantage of the TC cell in water pollution work is its lack of sensitivity.

2 Ionization detectors

a Flame

This detector consists of a flame situated between a cathode and anode. As carrier gas alone burns, some electrons and negative ions are produced which are collected at the anode and recorded as baseline. When carrier gas plus sample component are burned, more electrons and negative ions are produced which result in a peak on the recorder. The detector is capable of "seeing" nanogram quantities of organic compounds; however, the detector is sensitive to all organic compounds. This lack of specificity produces disadvantages in the analysis of water extracts which contain a variety of naturally occurring organics.

b Electron capture (See Figure 2)

This detector consists of a radiation source (e.g., tritium) capable of producing slow electrons in a carrier gas such as nitrogen. The electrons collected at the anode are recorded as baseline. When sample components which have an electron affinity (e.g., chlorinated hydrocarbons) enter the detector, electrons are "captured." The subsequent decrease in current is recorded as a peak. The detector has the advantage that it is extremely sensitive (picogram range) and is somewhat selective.

c Thermionic

A recent adaptation of the flame ionization detector shows promise for the specific analysis of organic phosphorus compounds. An alkali salt is incorporated into the design of a conventional flame ionization detector so that the salt heated by the flame produces an ion current. When compounds containing phosphorus emerge from the column, they give 600X the response with this detector as with the conventional flame.

3 Microcoulometric

Although less sensitive (by approximately a factor of 10) than electron capture.

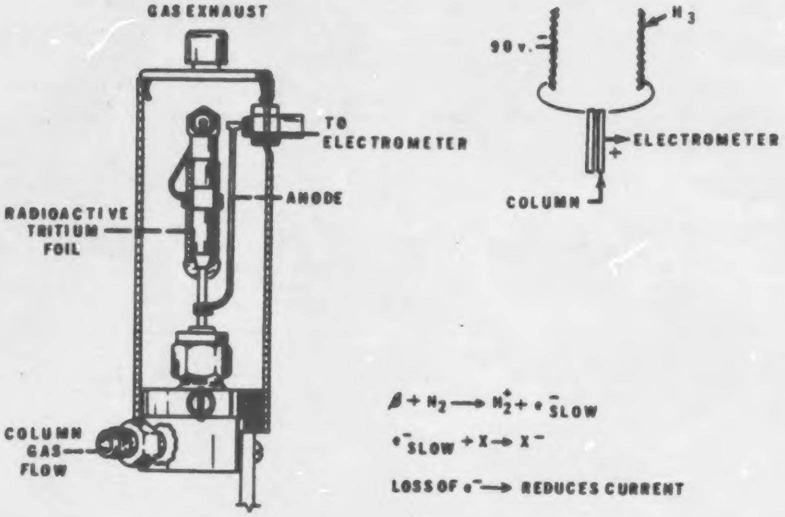

Figure 2. ELECTRON CAPTURE DETECTOR (Wilkens Instrument Company)

this detector is finding wide use in pesticide analysis. This highly specific detector consists of titration cells for the measurement of chloride-containing and sulfur-containing compounds. The sample component emerging from the column is combusted to produce HCl or SO_2, respectively. HCl is continuously titrated by silver ions present in the cell; the amount of current required to regenerate these silver ions is recorded as a peak. The system for sulfur containing compounds is analogous except that SO_2 produced is continuously titrated by I_2 which is subsequently regenerated.

Another microcoulometric detector has recently been applied to the specific determination of nitrogen. It is based on the reduction of nitrogen-containing compounds to NH_3 which is then titrated by H^+ in the titration cell.

4 Coulson Electrolytic Conductivity

This detector was primarily developed for the detection of organic halides, organic nitrogen compounds, and organic sulfur compounds. The unit consists of pyrolyzer with a separately heated inlet block, water circulating and purification system, detector cell and dc conductivity bridge. The sample is oxidized or reduced and reaction products form electrolytes when dissolved in the deionized water. Changes in conductivity between two platinum electrodes are measured by the dc bridge. (Figure 2 a)

E Recorder

The recorder system registers the response of the detector to sample components. In the case of ionization detectors, it is often necessary to employ an electrometer in order to amplify the small current changes.

Expensive integration and digital read-out equipment is also available to facilitate measurement of peak areas.

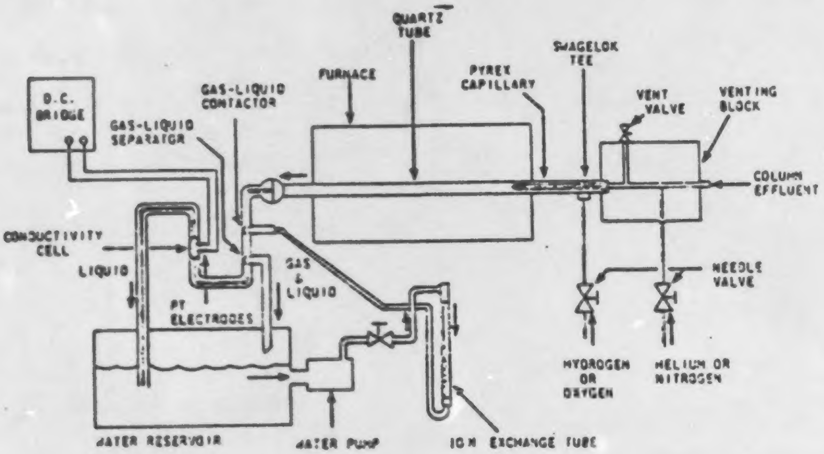
QUARTZ TUBE
SWAGELOK TEE
FURNACE
PYREX CAPILLARY
D.C. BRIDGE
GAS-LIQUID CONTACTOR
VENT VALVE
VENTING BLOCK
GAS-LIQUID SEPARATOR
COLUMN EFFLUENT
CONDUCTIVITY CELL
LIQUID
GAS & LIQUID
PT ELECTRODES
NEEDLE VALVE
HYDROGEN OR OXYGEN
HELIUM OR NITROGEN
WATER RESERVOIR
WATER PUMP
ION EXCHANGE TUBE

Figure 2 a. FLOW DIAGRAM OF ELECTROLYTIC CONDUCTIVITY DETECTOR (Coulson Instruments Company)

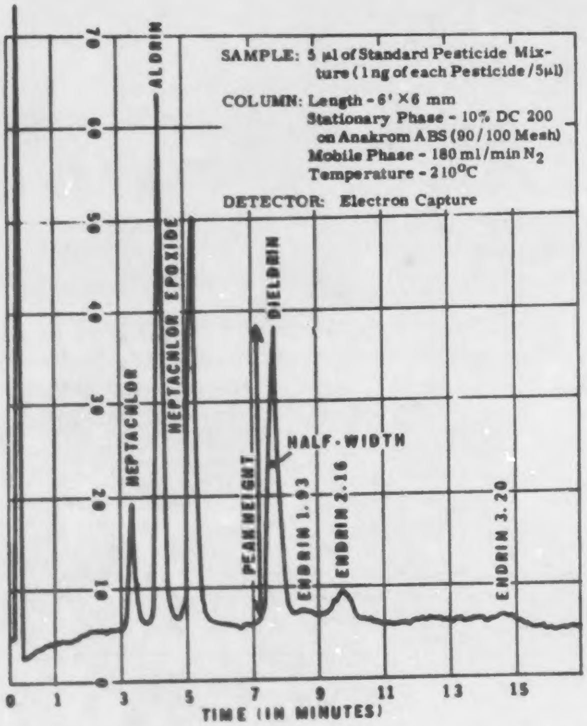

Figure 3. GAS CHROMATOGRAM OF PESTICIDE MIXTURE

III QUALITATIVE ANALYSIS

A Retention Time

The retention time of a sample component is defined as the time it takes for that component to travel through the column. There are a number of variables which affect the retention time of a compound.

1 Physical parameters of column operation

   a Column length

   b Column temperature

   c Carrier gas flow rate

2 The nature and amount of stationary liquid itself

For a given set of column conditions, a specific compound will have a specific retention time (See Figure 3 and Table 5). Various column and detector combinations can be used to confirm identification.

B Retention Volume

Retention volume is defined as the total volume of gas required to move a component through the column.

$$\text{RETENTION VOLUME } (R_V) = \text{RETENTION TIME } (R_T) \times \text{FLOW RATE}$$

C Relative Retention Times and Volumes

It is possible to interpret data more easily by reporting retention data relative to a particular compound (e.g., aldrin as in Table 5).

IV QUANTITATIVE ANALYSIS

A Measurement of Peak Area

The quantity of sample component present is directly proportional to the area under its peak. (NOTE: This assumption can only be made if it has been previously determined that a linear response is obtained in the range under study.) The following are a few of the ways in which this area can be measured.

1 Planimeter

2 Triangulation

3 Peak height × half-width (see dieldrin peak in Figure 3)

AREA = peak height × peak half-width

4 Disc integrator

B Measurement of Peak Height

With the electron capture detector it may be possible to use peak height for quantitative measurements where the following conditions are met.

Table 5. RETENTION DATA FOR FIGURE 3

Pesticide	Retention Time (R_T)	Relative Retention Time
Heptachlor	3.3 minutes	0.79
Aldrin	4.2	1.00
Heptachlor Epoxide	5.3	1.26
Dieldrin	7.7	1.84

1 A steady basline is obtained.

2 Retention times can be reproduced from one injection to the next.

V SUMMARY

The basic components of a gas chromatograph have been described. Elementary aspects of quantitative and qualitative analysis are presented.

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Descriptors: Adsorbents, Chromatography, Gas Chromatography, Organic Matter, Separation Techniques, Water Analysis

INFRARED INSTRUMENTATION

I THEORY

A The Effect of Infrared Radiation on the Molecule

1 All molecules are made up of atoms connected by chemical bonds which act very much like springs.

2 Atoms or atomic groups are in continuous motion with respect to each other. Each molecule has specific vibrational frequencies for these motions.

3 A molecule which has the same vibrational frequency as the infrared radiation striking it will absorb that energy.

4 If the radiant frequency differs from the characteristic frequencies of the molecule, the radiation passes through the molecule undiminished.

5 The characteristic frequencies for particular molecules are determined by the masses of their atoms, their spatial geometry and the strength of their connecting bonds.

B Measuring Infrared Radiation

1 The infrared spectrum gives basic information about the molecular structure of a compound.

2 Organic chemicals are largely made up of different combinations of atomic building blocks called functional groups: $-OH$, $-NH_2$, $-CH_3$, $-CO$, $-CN$, $-\overset{|}{\underset{|}{C}}-O-\overset{|}{\underset{|}{C}}-$, $-COOH$.

3 These subgroups have characteristic absorption bands in certain parts of the infrared spectrum.

4 Spectra correlation charts provide a key to the location of characteristic absorption bands of the most common functional groups.

II THE INSTRUMENT

A Source

1 A length of ceramic tubing heated to about 1200°C

2 It produces a continuous spectrum of radiant energy, most of which falls within 2. 5 to 15 micron region.

B Monochromator

1 It disperses the radiation into its component wavelengths.

2 Selects the particular wavelength interval transmitted to the detector.

3 Maintains, by varying slit widths, approximately constant energy through the slits to the detector.

C Wavelength

1 A mirrors rotational position determines the particular wavelength which emerges from the slit.

2 Linkage with the recorder drum shaft insures that the wavelength scale on the drum corresponds exactly to the wavelength selected.

D Resolution

1 A NaCl prism provides the best combination of dispersion and transmission in the operating range.

2 The sealed and desiccated monochromator protects the prism from moisture and dust.

E Thermocouple

1 A high sensitivity thermocouple consists of a blackened gold leaf target welded to two pins.

2 Temperature differences at the junctions of the leaf and pins set up a thermoelectric potential which is utilized.

F Optical Attenuator

1 By subtracting energy from the reference beam, the attenuator makes the amount of energy in the reference beam precisely the same as that in the sample beam.

2 This permits the pen to record directly in terms of sample transmittance.

G Instrument Performance

1 Scanning time

Twelve minutes for total scan, automatically programmed for constant rate per spectral slit width for constant accuracy over the wavelength range.

2 Accuracy

a Abscissa ± 0.03μ, ordinate ± 1%T.

b Less than 3% scattered light at 14.7μ and less than 1% from 2.5μ to 14μ.

III MICRO INFRARED TECHNIQUE

A Solution Technique

1 The technique most reliable for quantitative measurements in infrared region is the solution technique.

2 One drawback to this technique is the scarcity of suitable solvents with sufficient areas of transparency.

3 Carbon disulfide CS_2 is the most valuable of these because of its transparency in the "finger print" region of the infrared from 1,350 to 650 cm^{-1} (7.4μ to 15). Carbon tetrachloride is a useful solvent from 4,000 to 1,650 cm^{-1} (2.5μ to 6.0μ).

4 The sample is dissolved in 0.3 ml of solvent. The solution is then transferred to a 5 mm energy-path cavity cell by a hypodermic syringe. A matching cavity cell is used for solvent compensation.

5 With this technique, sensitivities down to 5-10 μg of strongly absorbing compounds are achieved without resorting to scale expansion.

B The Potassium Bromide KBr Pellet Technique

1 Since KBr is transparent throughout the commonly used portion of the infrared region, the entire infrared region is available as contrasted to the use of solvents.

2 A 6X beam-condenser is used to increase the energy transmitted through the pellet.

3 By this procedure a reliable sensitivity of about 5μg can easily be attained without scale expansion.

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This outline was prepared by J.W. Mandia, Chemist, formerly with National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Infrared Radiation, Instrumentation, Water Analysis

INFRARED

Procedure For Use Of Infracord

1. The "on-off" toggle switch should be in the "on" position, and the instrument warmed up for about 15 minutes.

2. The "reset-stop-scan" knob should be in the "reset" (extreme counter-clockwise) position; never try to manually move the chart drum unless this knob is in the "reset" position.

3. Please do not touch the "slit" or "100%" knobs, or those on the side of, or in the rear of, the instrument.

4. The pen holder assembly should be drawn back so that the pen is not touching the chart drum.

5. With the "reset-stop-scan" switch in the "reset" position, gently remove the chart drum from the instrument.

6. Place a piece of recorder paper on the drum in such a way that the guidelines above and below the hole in the upper left corner of the paper are aligned with the line inscribed in the drum itself. Replace the drum in the instrument.

7. Gently move the pen holder assembly forward so that the tip of the pen touches the paper.

8. Lay the two sodium chloride salt plates provided on a tissue. Always hold the sodium chloride salt plates with a tweezer or the finger tips; in the latter case, hold the plates by the ends, or narrow sides. Never hold the plates by the wide sides since these are the optical faces.

9. Dip the end of a capillary tube into the liquid sample to a depth of 1-2 mm. The liquid will rise in the tube via capillary action.

10. Holding the capillary tube containing the sample in a vertical position, touch it lightly several times to the center of a salt plate; a small spot of sample will be formed.

11. Place the second salt plate on top of the first plate and press down lightly; the spot should spread out so as to cover almost the entire area between the two plates.

12. Remove the spring clip holder from the instrument and place the two-plate "sandwich" in it.

13. Place the holder in the sample beam (front) bracket on the instrument.

14. Move the "reset-stop-scan" knob to the scan position; the rest of the process is automatic and the drum will stop revolving when the spectrum of the sample has been completed (about 13 minutes).

15. Draw the pen holder assembly back from the chart paper.

16. Move the "reset-stop-scan" knob to the "reset" position and slowly rotate the drum in a counter clockwise direction until it stops.

17. Remove the drum from the instrument, and then, the chart paper from the drum.

18. Place a new piece of chart paper on the drum and place the drum back in the instrument.

19. Remove the spring clip holder from the instrument.

20. Remove the salt plat "sandwich" from the holder and separate the two plates.

21. Using a tissue dipped in the beaker of solvent, gently, but thoroughly, wipe the sample from both of the salt plates; allow the plates to air dry.

This outline has been prepared by Charles R. Feldmann, Chemist. National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

TOTAL CARBON ANALYSIS

I INTRODUCTION

A History of Carbon Analyses

In the wake of a rapid population growth, and the increasing heavy use of our natural waterways, the nation, and indeed the world, is presented with the acute problem of increased pollutional loads on streams, rivers and other receiving bodies. This has resulted in a growing awareness of the need to prevent the pollution of streams, rivers, lakes and even the oceans. Along with this awareness has developed a desire for a more rapid and precise method of detecting and measuring pollution due to organic materials.

B The Methods

In the past, two general approaches have been used in evaluating the degree of organic water pollution.

1 The determination of the amount of oxygen or other oxidants required to react with organic impurities.

2 The determination of the amount of total carbon present in these impurities.

C Oxygen Demand Analyses

The first approach is represented by conventional laboratory tests for determining Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). One of the principal disadvantages of these tests is that they are limited primarily to historical significance, that is, they tell what a treatment plant had been doing, since they require anywhere from two hours to five days to complete. Since up to now no faster method has been available, traditional BOD and COD determinations have become accepted standards of measure in water pollution control work even though they are essentially ineffective for process control purposes.

Until the introduction of the Carbonaceous Analyzer, all methods taking the second approach, the total carbon method of evaluating water quality, also proved too slow.

II THE ANALYSIS OF CARBON

A Pollution Indicator

Now the carbonaceous analyzer provides a means to determine the total carbon content of a dilute water sample in approximately two minutes. With proper sample preparation to remove inorganic carbonates, the instrument determines the total organic carbon content in the sample.

B Relationship of Carbon Analysis to BOD and COD

This quantity varies with the structure from 27 percent for oxalic acid through 40 percent for glucose to 75 percent for methane. The ratio of COD to mg carbon also varies widely from 0.67 for oxalic acid through 2.67 for glucose to 5.33 for methane. Representative secondary sewage effluents have given a ratio of COD to carbon content of between 2.5 and 3.5 with the general average being 3.0.

The BOD, COD and carbon contents of these and some other representative compounds are summarized in the following table.

Sample	5-Day BOD-mg/mg	COD-mg/mg	% Carbon
Stearic Acid - $C_{18}H_{36}O_2$	.786	2.91	76
Glucose - $C_6H_{12}O_6$	.73	1.07	40
Oxalic Acid - $C_2H_2O_4$	.14	.18	27
Benzoic Acid - $C_7H_6O_2$	1.38	1.97	69
Phenol - C_6H_6O	.05 to 2.1 depending upon concentration	2.36	77
Potassium Acid Phthalate $KHC_8H_4O_4$	.95	1.15	47
Salicylic Acid - $C_7H_6O_3$	1.25	1.60	61
Secondary Effluent, Clarified	13*	75*	21*
" " "	23*	67*	12*
" " "	4*	36*	7*

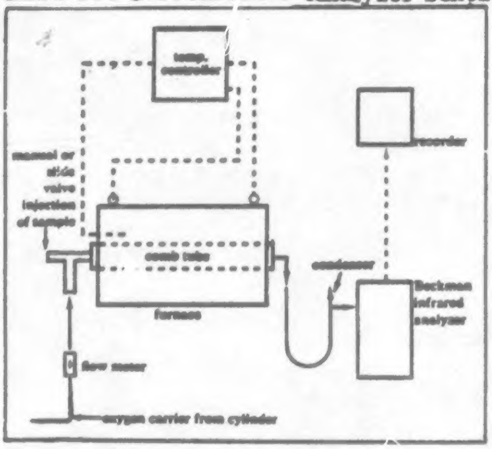
*In units of mg/l

III THE INFRA-RED TYPE CARBON ANALYZER

A Principle of Operation

Basically the infra-red carbonaceous analyzer made by Beckman, consists of three sections - a sampling and oxidizing system, a Beckman Model 315 Infrared Analyzer and a strip-chart recorder.

Infra-red Carbonaceous Analyzer Schematic


temp. controller
recorder
manual or slide valve injection of sample
comb tube
furnace
condenser
Beckman infrared analyzer
flow meter
oxygen carrier from cylinder

V

A micro sample (20-40 μl) of the water to be analyzed is injected into a catalytic combustion tube which is enclosed by an electric furnace thermostated at 950°C. The water is vaporized and the carbonaceous material is oxidized to carbon dioxide (CO_2) and steam in a carrier stream of pure oxygen or CO_2-free air. The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and the condensate removed. The CO_2, oxygen or air, and remaining water vapor enter an infrared analyzer sensitized to provide a measure of CO_2. The output of the infrared analyzer is recorded on a strip chart, after which, the curve produced can be evaluated by comparing peak height with a calibration curve based upon standard solutions. Results are obtained directly in milligrams of carbon per liter.

B Application

Results(1) show that the method is applicable for most, if not all, water-soluble organic compounds -- including those that contain sulfur, nitrogen, and volatiles.

Nonvolatile organic substances can be differentiated from volatiles, such as carbon dioxide or light hydrocarbons by determination of carbon both before and after the sample solution has been blown with a carbon-free gas.

C Sample Preparation

The Carbonaceous Analyzer is often referred to as a total carbon analyzer because it provides a measure of all the carbonaceous material in a sample, both organic and inorganic. However, if a measure of organic carbon alone is desired, the inorganic carbon content of the sample can be removed during sample preparation.

1 Removal of inorganic carbon

The simplest procedure for removing inorganic carbon from the sample is one of acidifying and blowing. A few drops of HC1 per 100 ml of sample will normally reduce pH to 2 or less, releasing all the inorganic carbon as CO_2. Five minutes of blowing with a gas free of carbon sweeps out the CO_2 formed by the inorganic carbon. Only the organic carbon remains in the sample and may be analyzed without the inorganic interference.

2 Volatile carbonaceous material

Volatile carbonaceous material that may be lost by blowing is accounted for by using a dual channel carbon analyzer. Beckman's new analyzer has the previously detailed high temperature (950°C) furnace plus a low temperature (150°C) one. Using quartz chips wetted with phosphoric acid, the low temperature channel senses only the CO_2 (freed by the acid) in the original sample. The remaining organics and water are retained in the condenser connected to this low temperature furnace. None of the organics are oxidized by the 150°C furnace.

By injecting a sample into the low temperature furnace, a peak representing the inorganic carbon is obtained on the strip chart. Injecting a nonacidified sample into the high temperature furnace yields a peak representing the total carbon. The difference between the values determined for the two peaks is the total organic carbon.

3 Dilute samples

If the sample is dilute (less than 100 mg/liter carbon) and is a true solution (no suspended particles) no further preparation is required.

4 Samples containing solids

If the sample contains solids and/or fibers which are to be included in the determination, these must be reduced in size so that they will be able to pass through the needle which has an opening of 170 microns (needles having larger openings may be obtained if necessary). In most cases, mixing the sample in a Waring Blender will reduce the particle size sufficiently for sampling.

IV PROCEDURE FOR ANALYSIS

A Interferences

Water vapor, resulting from vaporization of the sample, causes a slight interference in the method. Most of the water is trapped out by the air cooled condenser positioned immediately after the combustion furnace. However, a portion of the water vapor passes through the system into the infrared detector and appears on the strip chart as carbon. The water blank also appears on the standard calibration curve, and is therefore removed from the final calculation. In tests of solutions containing the following anions: NO_3^-. Cl^-, SO^{-2}, PO_4^{-3}, no interference was encountered with concentrations up to one percent.

B Precision and Accuracy

The recovery of carbon from standard solutions is 98.5 - 100.0 percent. The minimum detectable concentration using the prescribed operating instructions is 1 mg/1 carbon. Generally, the data are reproducible to ± 1 mg/1 with a standard deviation of 0.7 mg/1 at the 100 mg/1 level.

V THE FLAME IONIZATION TYPE CARBON ANALYZER

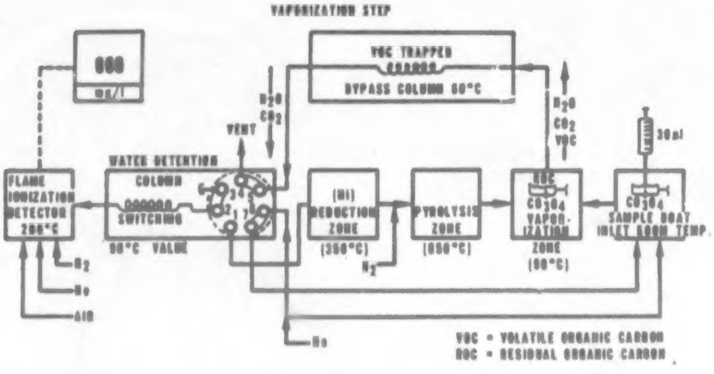
An example of a flame ionization carbonaceous analyzer is the one produced by Dohrmann.

To determine TOTAL ORGANIC CARBON a 30 μl acidified water sample is injected into a sample boat containing a cobalt oxide oxidizer at room temperature. The boat is then advanced to the 90° C vaporization zone where H_2O, CO_2 (from dissolved CO_2, carbonates and bicarbonates) and organic carbon materials which are volatile at 90° C are swept into the bypass column. Here volatile organic carbon (VOC) is trapped on a Porapak Q column at 60° C while the H_2O and CO_2 are swept through the switching valve and vented to atmosphere.

After sample vaporization, the valve is automatically switched to the pyrolyze position and the boat is then advanced to the pyolysis zone. Residual organic carbon (ROC) materials left in the boat react with the Co_3O_4 at 850° C to produce CO_2. At the same time the bypass column is backflushed at 120°C thus sweeping the VOC material through the pyrolysis zone. Both the VOC and the CO_2 (from the ROC) are swept by helium into the hydrogen enriched nickel catalyst reduction zone where all carbon is converted to methane at 350° C.

The reduction product is swept through the switching valve, the water detention column and into the flame ionization detector which responds linearly to methane. The detector output is integrated and displayed in milligrams per liter (mg/l) or parts per million (ppm) on the digital meter.

To determine total carbon, simply set the function switch to TOTAL CARBON, and cycle an unacidified sample through the vaporize and pyrolyze steps. The switching valve remains in the pyrolyze position, directing ALL carbonaceous matter to the detector.


VAPORIZATION STEP
888
mg/l
VOC TRAPPED
BYPASS COLUMN 60°C
H2O
CO2
VENT
H2O
CO2
VOC
30 μl
WATER DETENTION
COLUMN
SWITCHING
90°C VALVE
FLAME
IONIZATION
DETECTOR
280°C
(Ni)
REDUCTION
ZONE
(350°C)
PYROLYSIS
ZONE
(850°C)
ROC
Co3O4
VAPORIZATION
ZONE
(90°C)
Co3O4
SAMPLE BOAT
INLET ROOM TEMP.
H2
He
AIR
H2
He
VOC = VOLATILE ORGANIC CARBON
ROC = RESIDUAL ORGANIC CARBON

VI THE AUTOCLAVE TYPE OF CARBON ANALYZER

Oceanography International makes a TOC apparatus in which the sample is placed in an ampule that contains phosphoric acid and potassium persulfate. The ampule is flame sealed and autoclaved. The tip of the ampule is then broken and the CO_2 removed by a gas stream that carries the CO_2 from the sample to an infrared detector. The autoclave digestion approximates a COD in which the COD's H_2SO_4 is replaced with H_3PO_4 and $K_2Cr_2O_7$ is replaced by $K_2S_2O_8$. This instrument does not have difficulty with salts coating a catalyst, but has a comparatively high time requirement.

VII LOW ORGANIC CARBON LEVELS

The organic carbon in solution can be measured down to levels as low as 50 ppb using an instrument manufactured by Barnstead. Samples up to 100 ml in volume are introduced into the instrument. The sample is introduced into the system with a syringe or pipet. Inorganic carbon in the form of dissolved CO_2 is first stripped out of the sample by a stream of air (acidification is used to bring the pH to 4.2). The air carries the inorganic carbon (CO_2) to a measuring chamber containing 18 megohm-cm, zero-organic water. The CO_2 dissolves in the pure water and the resultant change in specific resistance is measured and stored in memory. See the figure on page 17-6.

Following the determination of inorganic carbon, the ultra-violet lamp within the irradiation chamber is energized. All dissolved organic compunds - both volatile and non-volatile - are completely oxidized. The resulting CO_2 is carried by the air stream to the measuring chamber where it also dissolves in the water. The unit again measures the resistivity of the water within the measuring chamber to determine the total carbon of the sample. The system now automatically subtracts the stored inorganic carbon value from the total carbon value and displays the difference, which is the level of organic carbon in the sample.

After the result has been displayed, the unit automatically begins a clean-up cycle in which all CO_2 and other contaminants in the system are removed by an ion-exchange cartridge. Once all water within the system has returned to an 18 megohm-cm, zero-organic condition, the system is ready for the next sample.

Dohrmann has a ultra-violet lamp system for low organic carbon levels that connects along side of their regular analyzer. The CO_2 from the ultra-violet lamp component passes to the regular analyzer and its flame ionization detector.

Ultra-violet systems will give low results if the organic carbon is not in solution.

VIII APPLICATIONS

Several of the many research and industrial applications of the Carbonaceous Analyzer are listed below:

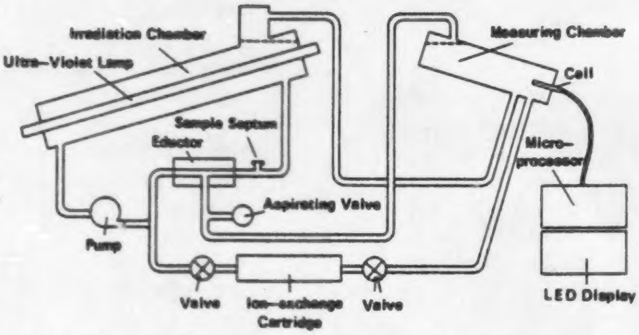
A Determine the efficiency of various waste-water renovation processes, both in the laboratory and in the field.

B Compare a plant's waste outlet with its water inlet to determine the degree of contamination contributed.

C Monitoring a waste stream to check for product loss.

D Follow the rate of utilization of organic nutrients by micro-organisms.

E To detect organic impurities in inorganic compounds.


Irradiation Chamber
Ultra-Violet Lamp
Sample Septum
Eductor
Aspirating Valve
Pump
Valve
Ion-exchange Cartridge
Valve
Measuring Chamber
Cell
Micro-processor
LED Display

Ultra-violet low organic carbon Barnstead apparatus.

A summary of some TOC laboratory instrument:-

Instrument Manufacturer	Mode of Oxidation	Detector
Beckman	950°C furnace and catalyst of Co oxides	Infra-red
Oceanography International	H_3PO_4-$K_2S_2O_8$ plus autoclave digestion	Infra-red
Astro, sold by Curtin Matheson	850°C combustion chamber	Infra-red
Dohrmann-Envirotech	850° furnace plus reduction to methane	Flame ionization
	(Low Organic Carbon Levels)	
Dohrmann-Envirotech	Ultra-violet lamp, H_3PO_4	Returned to unit with flame ionization
Barnstead	Ultra-violet lamp, H_3PO_4	Conductivity

Note that there may be other manufacturers of TOC equipment.

IX ADVANTAGES OF CARBON ANLYZER

A Speed

The Carbonaceous Analyzer's most important advantage is its speed of analysis. One analysis can be performed in 2 - 3 minutes for a channel on the Beckman instrument or double that on the Dohrman. This speed of analysis brings about economy of operation. This is probably more than the number of COD or BOD tests that can even be started, much less completed, in the same period of time.

B Total Carbon

Another advantage is that the measure of carbon is a total one. The oxidizing system of the analyzer brings about complete oxidation of any form of carbon. No compound has been found to which the method is inapplicable.

X CONCLUSIONS

The Carbonaceous Analyzer provides a rapid and precise measurement of organic carbon in both liquid and air samples. It should be found useful for many research and industrial applications, a few of which have been mentioned.

Because of its rapidity it may be found more useful than the more time-consuming BOD and COD measurements for monitoring industrial waste streams or waste treatment processes.

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This outline was prepared by Robert T. Williams, Chief, and revised by Charles J. Moench, Jr., Waste Identification and Analysis Section, MERL, USEPA, Cincinnati, Ohio 45268.

Descriptors: Biochemical Oxygen Demand, Carbon, Chemical Analysis, Chemical Oxygen Demand, Organic Matter, Organic Wastes, Water Analysis, Instrumentation, Nutrients

SPECIAL INSTRUMENTAL METHODS - MASS SPECTROMETRY AND NMR

I INTRODUCTION

Mass spectrometry and nuclear magnetic resonance spectrometry (NMR) are now widely used in organic analytical chemistry, but have only begun to find application to the analysis of chemical pollutants in the aquatic environment. Now that the cost, and in some cases the complexity of these instruments have decreased, they will undoubtedly become quite important in water pollution analysis during the next few years. This is especially true of mass spectrometry, and the majority of this lecture time will be devoted to examples of applications of this instrumental method to water pollution problems encountered at the Southeast Water Laboratory. Examples of NMR applications will also be given, as well as a brief discussion of the cost and manpower requirements, sample requirements, operating principles, and informational output of both methods. Inorganic mass spectrometry, a very useful method for elemental analysis of water samples, will not be discussed since the operating techniques and sample requirements are quite different from those for organic mass spectrometry.

II MASS SPECTROMETRY

A Economic factors

1. Mass spectrometers range in price from \$20, 000 to \$200, 000 or more. Ordinary general purpose instrumentation begins at between \$30, 000 and \$35, 000.
2. Mass spectrometers are complicated instruments and generally are "down" from 10% to 50% of the time, depending upon the make and complexity of the spectrometer. Maintenance requires money for parts and service.
3. Time is an important economic factor. To operate, maintain, and interpret spectra from a mass spectrometer of medium complexity with a reasonable work load, at least one trained person is required full time.

B Sample requirements

1. Solid samples can be handled by direct probe accessories. From 10^{-12} to 10^{-7} grams of sample are required, depending upon sample volatility and instrument sensitivity. Much information can be obtained from a sample of low purity, say 90%, and some useful information can be obtained from simple mixtures. Viscous liquids are also generally handled in the direct probe.
2. The liquids sample inlet on a mass spectrometer is generally used for volatile liquids. Sample size is between 0. 1 and 1 mg, and, here again, much information can be obtained from impure samples or in certain cases, samples in solution.
3. Gaseous samples are handled in special gas sampling flasks and inlets.
4. Gas chromatographic inlet system

   The interfacing of a gas chromatograph to a mass spectrometer provides a very useful sample introduction mode. Using this technique, any compound that will chromatograph is eluted from the GC column, passes through a special sample enricher, and goes into the mass spectrometer proper. The enricher, known as a separator, removes the GC carrier gas preferentially, thus increasing the concentration of the eluted sample compound while lowering the pressure to a level suitable for the mass spectrometer. The application of this GC introduction mode to water pollution analysis is obvious. An organic extract of a water sample is chromatographed under previously established conditions, and a rapid scan (1 to 3 seconds) is made of each eluting component of the extract to produce the corresponding mass spectrum. Sample size required here is usually on the order of 0. 1 μg of each component, although useful spectra can sometimes be obtained with nanogram quantities.

C Operating principles

Regardless of which sample introduction mode is used, the sample must be in the gaseous state for the process of ionization. The sample molecules are bombarded by a beam of electrons of variable energy in the ion source (Figure 1) to produce positive

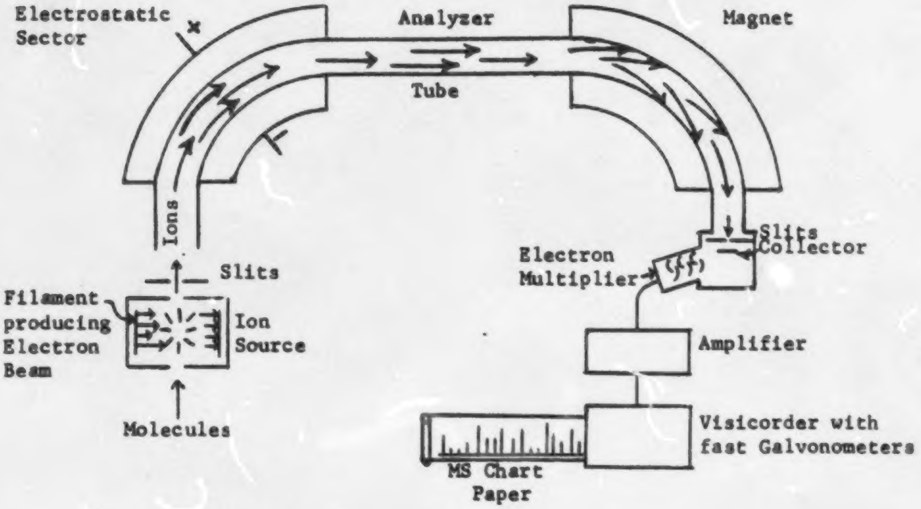
Electrostatic Sector
Analyzer
Tube
Magnet
Ions
Slits
Filament producing Electron Beam
Ion Source
Molecules
Slits
Collector
Electron Multiplier
Amplifier
Visicorder with fast Galvonometers
MS Chart Paper

Figure 1. Block diagram of a double-focusing mass spectrometer.

ions. These ions are swept through a series of slits and down the analyzer tube by a strong electrostatic field. The source and tube must be maintained at a low pressure (<10^{-5} torr) to minimize collisions of the ions with each other or with air molecules. The ions pass through a magnetic field where they are deflected to different degrees based on their mass to charge ratios. By sweeping the magnetic field strength, ions of successively increasing mass are sequentially brought into focus at the collector. In a double-focusing mass spectrometer, resolution is improved by an electrostatic sector which renders the ion beam monoenergetic by velocity focusing before it arrives at the magnetic focusing sector. The electron multiplier amplifies the signal received at the collector and the resulting signal is further amplified to drive a set of galvonometers which make a trace on a photographic chart paper.

D Information output

1 All fragment ions of a molecule give rise to peaks of certain intensities with positions on the abscissa of the spectrum corresponding to ion masses (m/e values). This information constitutes the mass spectrum, and comparison with the spectra of known compounds give definitive compound identification. Figure 2 is a plot of a typical mass spectrum, that of bis-(2-ethylhexyl) phthalate, compared with that of a compound extracted from the Tennessee River. They are practically identical.

2 The molecular weight of a compound can often be obtained from its mass spectrum. The fragment ion of greatest mass in most cases corresponds to the molecular weight of the compound (Figure 2, M^+=390), having been created by loss of only one electron during electron bombardment.

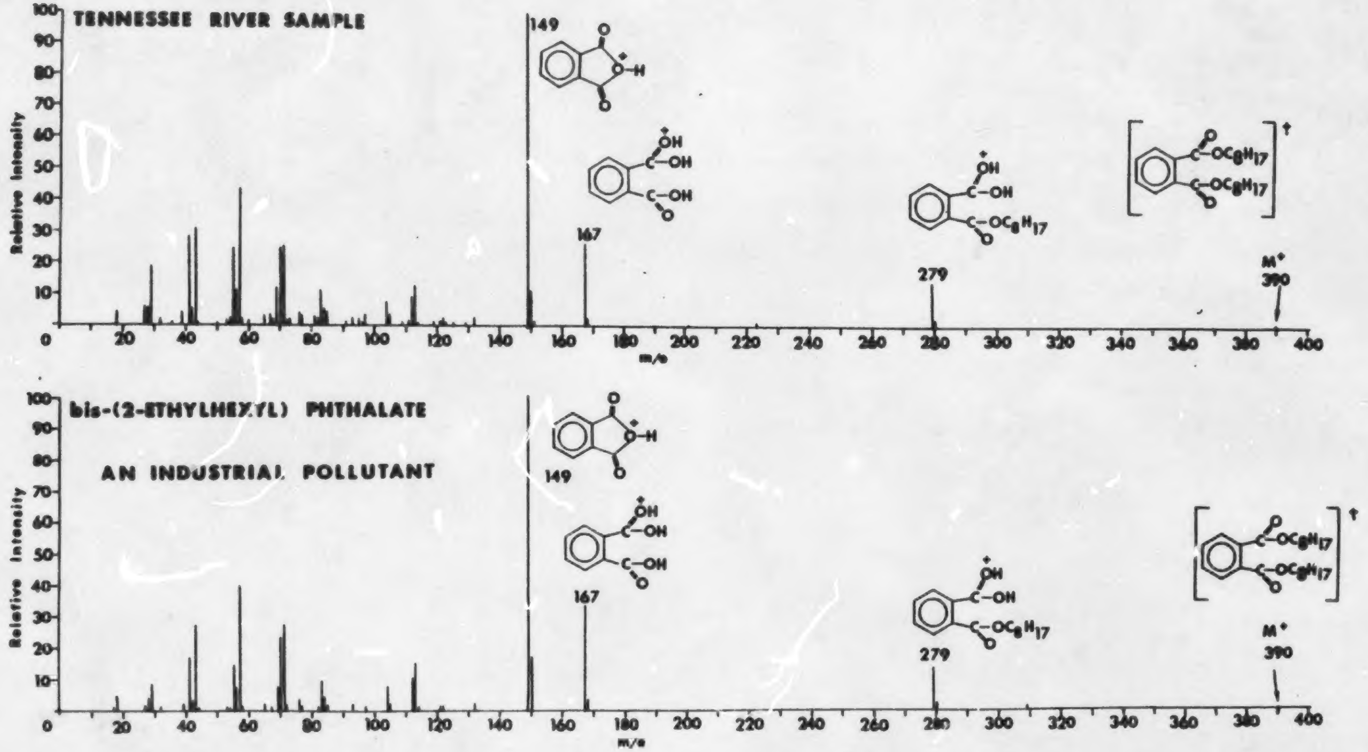
TENNESSEE RIVER SAMPLE
Relative Intensity
100
90
80
70
60
50
40
30
20
10
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
m/e
149
167
279
M+
390
OC8H17
OH
bis-(2-ETHYLHEXYL) PHTHALATE
AN INDUSTRIAL POLLUTANT

Figure 2

196

3 The masses of important fragment ions indicate the empirical formulae of the ions and hence give clues as to structures of parts of the molecule (Figure 2).

4 Isotope peaks are helpful in determining if a molecule contains chlorine, bromine, sulfur, mercury, and other atoms which have more than one highly abundant naturally occuring isotope.

5 High resolution mass spectrometry is capable of providing almost the exact mass (to four decimal places) of the parent ion and of the important fragment ions from the molecule. This degree of accuracy of mass determination eliminates all but one or two possible empirical formulas for these ions, thus providing very important clues to the molecular structure.

III EXAMPLES OF APPLICATIONS OF MASS SPECTROMETRY TO WATER POLLUTION PROBLEMS

(Corresponding spectra will be projected and discussed.)

A The identification of S, S, S-tributyl phosphorotrithioate in Charleston Harbor, S. C., depended to a large extent on molecule weight determination by mass spectrometry.

B The identification of phenyl mercuric chloride, believed to be the cause of a fish kill in Boone Lake, Tennessee, involved the observation of mercury and chlorine isotope peaks in the mass spectrum.

C Diazinon was separated from a Tombigbee River water extract by thin layer chromatography, removed from the adsorbent, and analyzed by mass spectrometry.

D The analysis of petroleum rocket fuel residues extracted from water illustrate the applicability of mass spectrometry to oil pollution analysis.

E Bis-2-(ethylhexyl) phthalate was identified as a pollutant in the Tennessee River with the aid of mass spectrometry (Figure 2).

F An actinomycetes metabolite, responsible for earthy taste and odor problems in an Ohio municipal water supply, was identified as 2-methylisoborneol. The mass spectral fractionation pattern of the unknown compound was important in the elucidation of its structure.

G Nonylphenol has been extracted from a treated woolen mill effluent, collected upon elution from a gas chromatographic column, and identified by mass spectrometry using the direct probe accessory.

H Several terpenes and terpene derivatives in the treated effluent of a kraft paper mill have been identified by combination gas chromatography-mass spectrometry (GC-MS). The complexity of the gas chromatogram illustrates both the great problems encountered in waste characterization and the beauty of the GC-MS technique; whereby, many of the individual components can be identified.

I Three resin acids and several fatty acids have been identified in the effluent and in the foam downstream from a paper mill. The methyl esters of the acids were prepared by reaction with diazomethane, and the volatile esters were analyzed by the combined GC-MS technique.

IV NUCLEAR MAGNETIC RESONANCE SPECTROMETRY (NMR)

A Economic factors

1 NMR instrumentation ranges in price from about $20,000 for a "desk-top" low resolution (60 MHz) spectrometer to $85,000 for a 100 MHz instrument with common accessories.

2 NMR equipment is not as mechanically complex as mass spectrometry instrumentation; down time is generally only 10% to 20% and a full time operator is not necessary for a reasonable work load. However, an experienced operator is necessary for optimum output.

B Sample requirements

1 Conventional NMR samples must be in the liquid phase, either neat or in solution. Solvents must be deuterated or aprotic (e. g., CCl_4, CS_2) since any protons present absorb energy to give spectral interferences. Milligram quantities of sample (in solution) are required except that with particularly sensitive instrumentation 0. 1 mg can sometimes give good spectra, and with

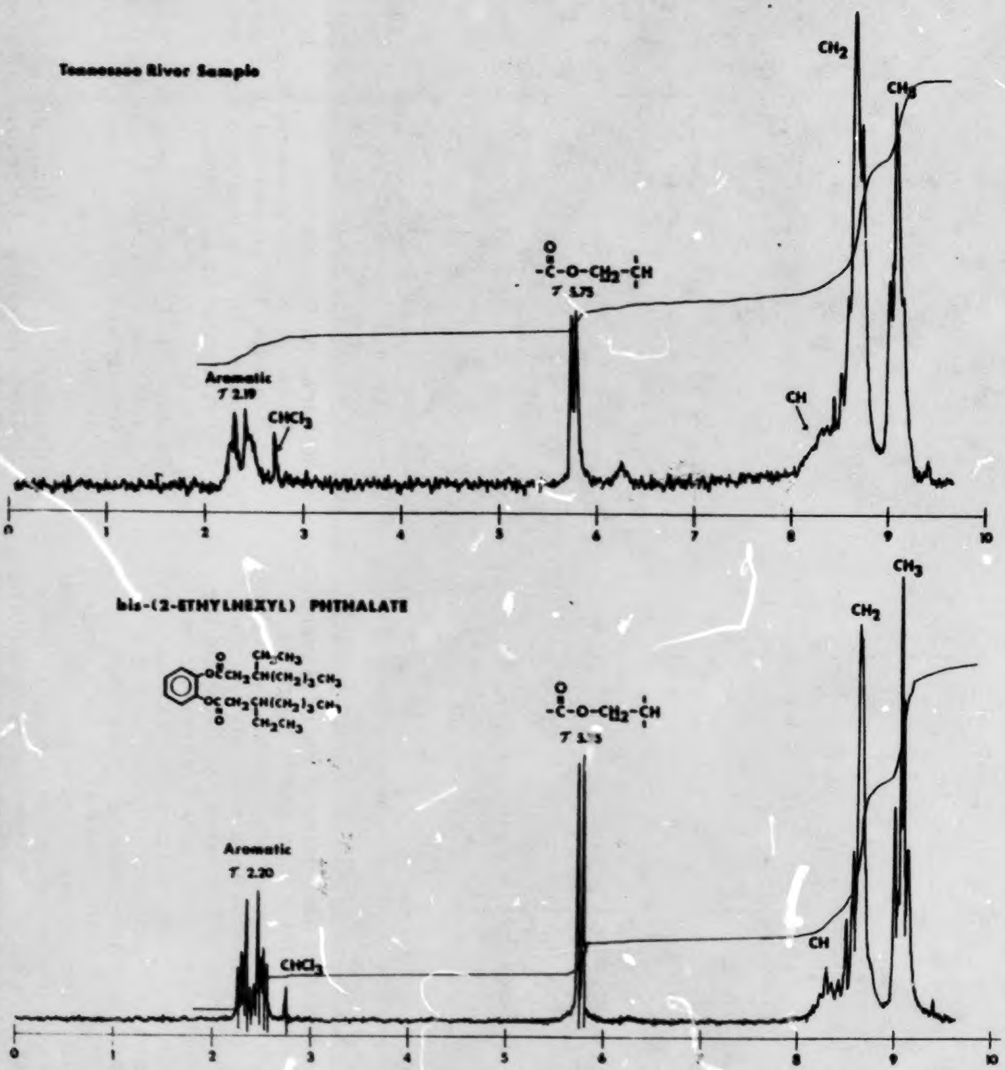
Tennessee River Sample
CH$_2$
CH$_3$
$-\overset{O}{C}-O-CH_2-CH$
τ 5.75
Aromatic
τ 2.19
CHCl$_3$
CH
0
1
2
3
4
5
6
7
8
9
10
bis-(2-ETHYLHEXYL) PHTHALATE
CH$_2$
CH$_3$
$-\overset{O}{C}-O-CH_2-CH$
τ 5.75
Aromatic
τ 2.20
CHCl$_3$
CH
0
1
2
3
4
5
6
7
8
9
10

Figure 3

time-averaging computerization to enhance the signal to noise ratio, less than 10 micrograms is sometimes sufficient. A purity of 90% is usually adequate to give useable spectra, and simple mixtures can often yield useful information.

2 A lack of sensitivity (a factor of 10^3 to 10^6 times as much sample is required as for mass spectrometry) and the longer time required for analysis are the principal disadvantages of NMR. The method is extremely valuable; however, in detailed structural elucidation, providing information as to the types and environments of hydrogen atoms which cannot be obtained by any other instrumentation.

C Information output

Hydrogen nuclei absorb energy in the radiofrequency range at certain frequencies depending on their environment. Thus, signals at certain frequency areas of the spectrum are characteristic of methyl, methylene, aldehydic, aromatic, etc., protons. (Figure 3). The NMR is capable of integrating these signal areas relative to each other; therefore, allowing calculation of the number of each type of proton in the molecule. In the case of simple mixtures, this feature often allows the calculation of percentage composition of the components.

In addition to absorbtion of radiofrequency energy, nuclei with magnetic moments, such as hydrogen, phosphorus, and carbon-13, interact with each other when in the same proximity to cause "splitting" of the proton signal peaks into a larger number of smaller peaks (Figure 3). This is known as "spin-spin coupling" and is a very informative element of the NMR method. Splitting rules allow the calculation of the number of peaks resulting from the coupling of one proton with other adjacent protons. Thus, the degree of splitting of a particular hydrogen signal is indicative of the protonic environment of the hydrogen atoms, and this allows important deductions to be made regarding the structure of the molecule.

D Examples of applications of NMR to water pollution problems

1 The NMR spectrum of a phthalate ester isolated from the Tennessee River provided the prime evidence for the detailed structure of the molecule, especially of the 2-ethylhexyl moities. It was later shown that this spectrum matched that of bis-(2-ethylhexyl) phthalate (Figure 3).

2 The NMR spectrum of the actinomycetes metabolite extracted from an Ohio municipal water supply showed the compound to have four tertiary methyl groups.

3 The frequency of the methylene absorption signals of the NMR spectrum of a toxic compound isolated from Charleston Harbor, S. C., indicated the compound to be the phosphate derivative of a phosphite ester present in an industrial effluent discharging into the harbor.

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This outline was prepared by Arthur W. Garrison, Research Chemist, Southeast Water Laboratory, WQO.

Descriptors: Chemical Analysis, Instrumentation, Mass Spectrometry, Nuclear Magnetic Resonance, Spectrophotometry, Water Analysis